H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 30 MARCH, 1961 No. 17

ANATOMIC EFFECTS OF CURLY TOP AND ASTER YELLOWS VIRUSES ON TOMATO[1, 2]

ENGRACIA ARGUELLES RASA[3] and KATHERINE ESAU[4]

INTRODUCTION

FIELD OBSERVATIONS indicate that it is difficult to distinguish tomato plants affected by curly top disease from those affected by aster yellows. Anatomic studies, previously carried out at Davis on flax plants affected with curly top and aster yellows (Girolami, 1955),[5] showed that the internal symptoms of these diseases are likewise not sharply differentiated. At the same time, it is well known that the two diseases are caused by distinct viruses transmitted by different species of leafhoppers. The present study was undertaken to increase our information on the anatomic and cytologic effects of curly top and aster yellows and to seek means of distinguishing between the two diseases in the tomato plant.

The anatomic changes induced by the curly top virus have been studied intensively in various hosts: in sugar beet (Artschwager and Starrett, 1936; Bennett and Esau, 1936; Esau, 1933, 1935*a*, *b*; 1957; Lackey, 1952); in tobacco (Bennett and Esau, 1936; Esau, 1941); in flax (Girolami, 1955); and to some extent in tomato (Esau, 1941). Much information is also available on the physiology of this virus (e.g., Bennett, 1934, 1937, 1943, 1944; Bennett and Esau, 1936). It is known to be a phloem-limited virus that moves through the plant at the same rates and through the same conduits as the food materials, and induces hyperplasia and necrosis in the phloem tissue.

The anatomic effects of aster yellows were studied in detail only on flax (*Linum usitatissimum* L.), by Girolami (1955). The aster yellows virus induces hyperplasia and necrosis in the phloem that differ only in detail from similar symptoms induced by curly top. The movement of aster yellows

[1] Submitted for publication September 19, 1960.

[2] Revised dissertation submitted by the senior author in June, 1960, to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[3] Formerly Research Assistant and graduate student in the Department of Botany, Davis.

[4] Professor of Botany and Botanist in the Experiment Station, Davis.

[5] See "Literature Cited" for citations referred to in the text by author and date.

virus in the plant has not been studied, but the similarity of phloem degeneration in plants affected by aster yellows and curly top suggests that the aster yellows virus is also a phloem-limited virus (cf. Esau, 1956).

MATERIAL AND METHODS

The original infected plants were obtained from Berkeley through the courtesy of Dr. J. H. Freitag of the Department of Entomology and Parasitology, University of California. These tomato plants (*Lycopersicon esculentum* Mill., Pearson variety) were inoculated by means of leafhoppers as the vectors. Curly top virus was transmitted by means of *Circulifer tenellus* (Baker) previously fed on sugar beets infected with curly top. Aster yellows virus was transmitted by means of *Macrosteles fascifrons* (Stål) previously fed on plantain infected with a Western strain of aster yellows. Further diseased material was obtained by grafting infected shoots upon healthy plants. This work was carried out in a greenhouse at Davis, California.

Curly top is easily transmissible to tomato by the use of leafhoppers, but the incidence of disease is comparatively low in aster yellows when the insect vector is used (cf. Smith, 1957, p. 40). Grafting is a more efficient method for transmission of aster yellows, and was successfully employed in the present study. Terminal and lateral buds from aster yellows plants were grafted to healthy plants which were two weeks old. To increase the uniformity in the comparison of the effects of the two viruses, curly top-diseased plants were also obtained by graft transmissions. The curly top plants died much sooner after infection than did the aster yellows plants. Therefore consecutive grafts of curly top had to be made in order to maintain the virus culture.

Terminal buds of both infected and control plants were removed at the time of grafting in order to induce lateral-bud formation. Collections of lateral shoots were made after the external symptoms became visible: two weeks, four weeks, and six weeks, consecutively, after grafting. The four-week collections were found to be most satisfactory for the comparative study because the anatomical symptoms of both diseases were well established at that time, and most shoot apices were still vegetative. At the later collections, flower buds were appearing; some flower primordia were present even in the collections made four weeks after grafting.

Plant parts were killed in Craf III, a chrome-acetic-formalin solution (Sass, 1958, p. 18), embedded in Tissuemat, and cut 10 microns thick. For the general study of the tissues, a progressive hematoxylin staining with safranin as counterstain was employed (Esau, 1944). For the study of callose and cytological details, such as slime bodies in sieve elements, a tannic acid-ferric chloride-resorcin blue combination was used (Cheadle *et al.*, 1953). Some material was stained with Giemsa stain according to the modified Bald method (Rawlins and Takahashi, 1952).

After some crystalline inclusions were discovered in the aster yellows tomato plants, certain other hosts of the virus were examined in infected condition: *Apium graveolens* L. (celery); *Callistephus chinensis* Nees (aster); *Linum usitatissimum* L. (flax); *Nicotiana rustica* L. (tobacco); *Plantago major* L. (plantain); and *Vinca rosea* L. (periwinkle). Some of

this material was examined in fresh condition, some in both fresh and killed and processed condition. When fresh material was used, parts of roots and shoots were squashed under the cover slip and treated with the desired solutions. Sometimes the squashes were stained with trypan blue, which differentiated the nucleus and the cell walls. The inclusion bodies were tested with HCl, H_2SO_4, chromic acid, picric acid, acetic acid, alcohol, and benzene as suggested by Garjeanne (1918).

THE HEALTHY PLANT

The Shoot Tip. Fourteen shoot tips from noninfected tomato plants (Pearson variety) were used to study the development of the shoot, with emphasis on the differentiation of the phloem tissue—the tissue that is primarily affected by the viruses of curly top and aster yellows diseases. The term shoot tip is used here to include the apical meristem and the youngest appendages, that is, a few leaves and inflorescence parts, if the latter were present.

The apical meristem of tomato has been described by Went (1944) and Bedesem (1958) as being dome-shaped. According to Bedesem (1958), the apical meristem has a mean diameter of 174 microns, and varies from 20 to 150 microns in height. The corresponding measurements in the present study were an average of 178 microns in width at the level of the most recently formed primordium, and 20 to 100 microns in height from that level to the apex. Bedesem stated that the age of the plant affects the width and height of the apical meristem, and that older plants have wider apices. This observation agrees with those made in the present study. As pointed out by Bedesem, the variation is at least in part dependent on plastochronic age; but it may also be related to the degree of activity of the meristem in producing flower primordia.

The apical meristem was not studied in detail, but was noted to vary in activity as expressed in the production of new primordia (compare figs. 1 and 2). Some of these may have been inflorescence rather than leaf primordia. In the following, reference is made simply to primordia without any attempt to identify them exactly. The structures definitely recognizable as leaves are so called.

Some shoot apices seemed to be inactive since no primordium was being initiated (fig. 1). In such shoots the youngest leaf was relatively large (leaf 1 in fig. 1, *B*) and was elevated above the shoot tip. In figure 1, *A*, the bulge on the right side below the apex is the base of the youngest leaf, which itself is not visible in this section. The leaf to the left is the second from the apex. Because of the low insertion of the first leaf, the shoot apex appears as a high dome. The notable feature of this dome is that the vacuolation of the pith extends above the youngest leaf. There is also some cortical vacuolation (fig. 1) so that the future vascular region is delimited—probably still as a residual meristem and not as procambium—above the youngest leaf.

Active shoots are illustrated in figure 2. The section in figure 2, *B*, was taken 140 microns below the apical meristem, one section above the insertion of leaf 1, and five sections below the insertion of a primordium (one similar to *a* in fig. 2, *A*). The position of the procambium of the primordium, in the

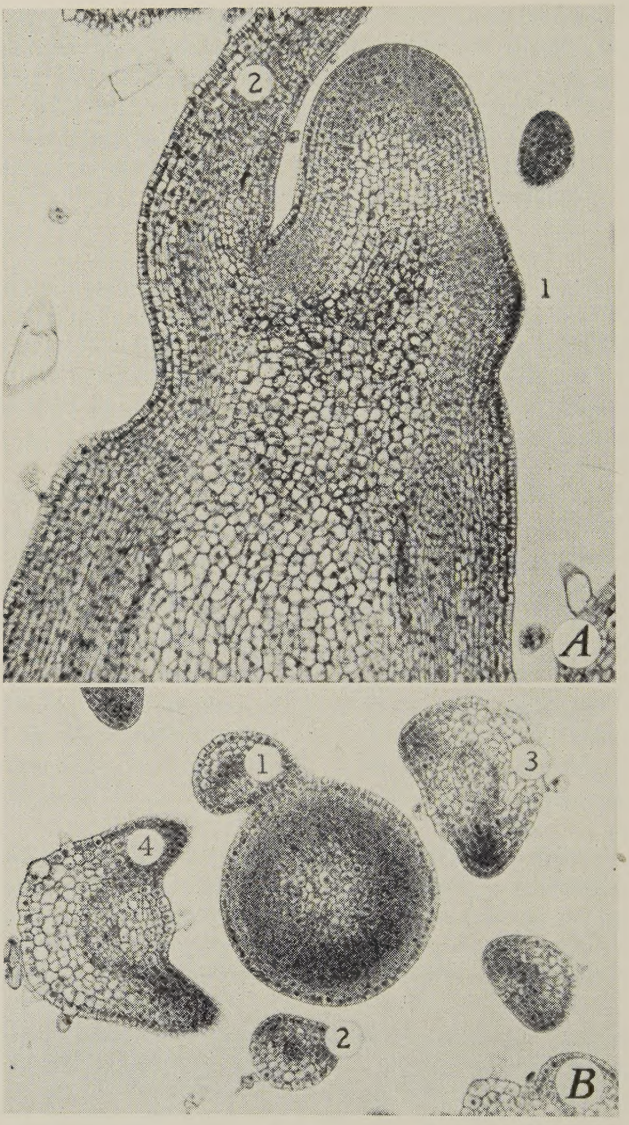

Fig. 1. Longitudinal (*A*) and transverse (*B*) sections of tomato shoot tips in which no primordia had been recently formed by the apical meristem. Numbers 1–4 indicate plastochronic ages of leaves. (Both × 120.)

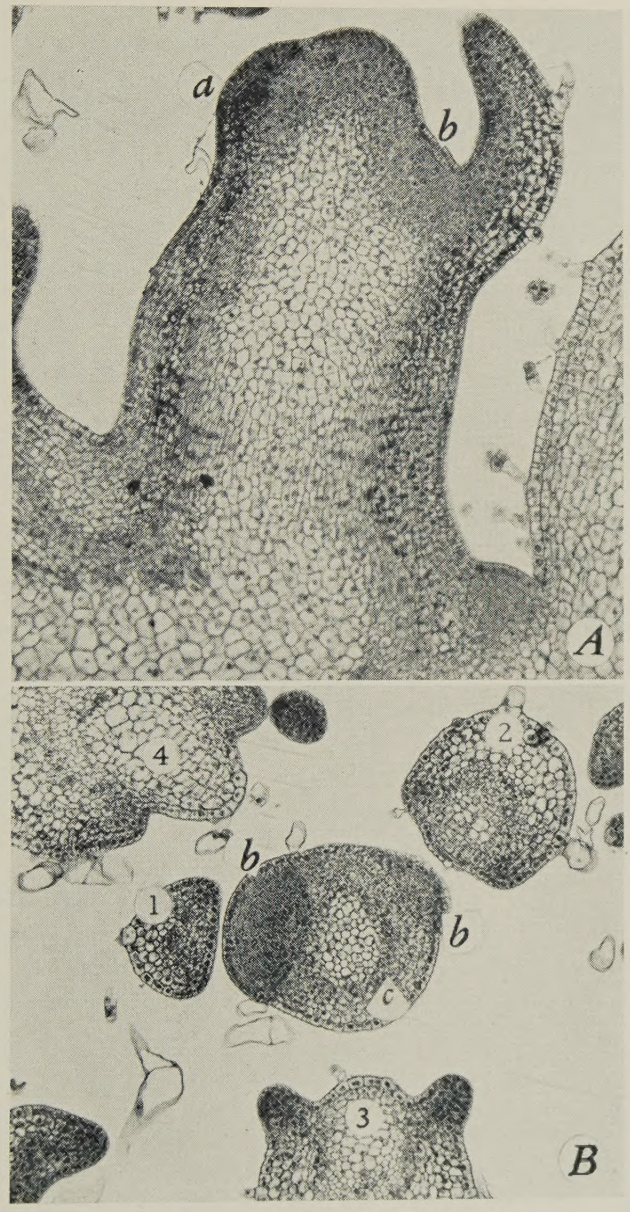

Fig. 2. Longitudinal (*A*) and transverse (*B*) sections of tomato shoot tips in each of which a primordium (*a*) had been recently formed by the apical meristem. *B* was cut below such primordium, but its vascular trace in form of procambium occurs at *c*. Numbers 1–4 indicate plastochronic ages of leaves. Details: *a*, recently formed primordium; *b*, axillary bud; *c*, trace procambium of primordium. (Both × 120.)

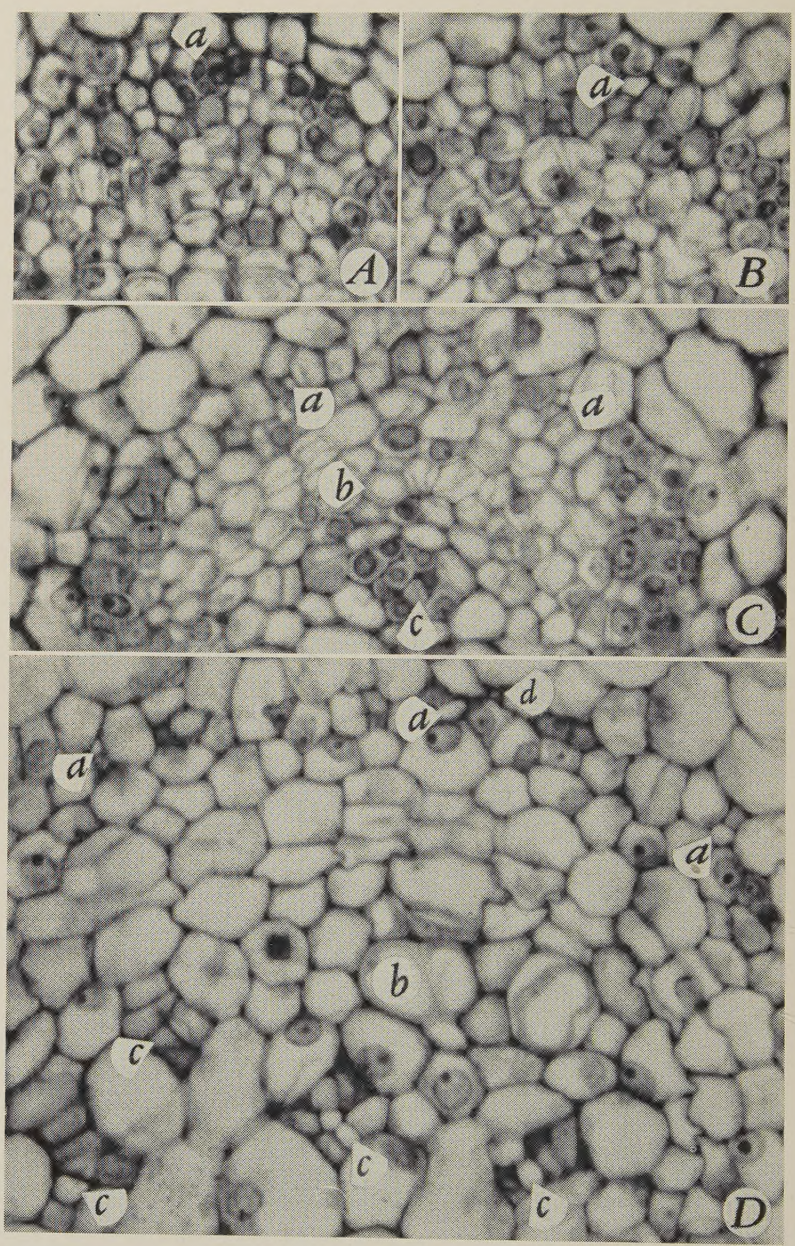

Fig. 3. Transverse views of vascular tissues in successively older leaves from a healthy tomato plant. Leaves 2 (*A*) and 5 (*D*) were taken from the same shoot as that shown in figure 2, *B*; leaves 3 (*B*) and 4 (*C*) were from another shoot. Details: *a*, sieve element of external phloem (immature in *A*); *b*, xylem element; *c*, sieve element of internal phloem (immature in *C*). (All × 280.)

stem, is marked *c* in figure 2, *B*. The lengths of leaves numbered 1, 2, 3, between the levels of their insertion on the stem and their apices were as follows: 140, 240, and 590 microns. The larger leaves were not measured. In contrast to the inactive shoot in figure 1, *A*, the active shoot in figure 2, *A*, has a low apical dome and a large primordium (*a*) on one side of the dome. The vacuolation of pith and cortex does not extend above the primordium.

Lateral buds are formed in the first and second leaf axils in an active shoot (fig. 2, *A*). According to Went (1944), such buds become flowers about the time 14 to 20 leaves have been initiated in the seedling. In Bedesem's (1958) material the reproductive stage was reached even earlier, when the plants had 10 leaves. The plants used in the present study were four, six, and eight weeks old. As stated under "Material and Methods," the apical meristem of the main axis was removed to induce lateral bud growth. In the four- and six-week collections, many of the lateral buds had formed several flower primordia. It thus appears that the active shoots were those entering the reproductive stage. The cause of inactive appearance of some of the shoots was not explored.

The leaves in tomato appear in spiral order some distance below the apical meristem. According to Hayward (1938), the tomato plant has a 2/5 phyllotaxy. Went (1944) wrapped a piece of thread around the stem of a tomato plant, following the nodes, and noted that after two complete circles had been made around the stem, leaf 1 was in the same vertical row as leaf 6, an evidence of a 2/5 phyllotactic pattern. In the material used in the present study, tomato leaves did approach a 2/5 arrangement but there was no straight-line relationship between leaves removed 5 plastochrons from one another; that is, leaves 1, 6, 11, etc., were arranged, not along an orthostichy (straight line), but a parastichy (a helix).

Plastochronic Sequence in Phloem Development in the Shoot. As a member of the Solanaceae, tomato has internal phloem. Another member of this family, tobacco, has been studied in detail by Esau (1938) with regard to the differentiation of the external and internal phloem. In general, phloem differentiation in tomato conforms to that in tobacco.

Cross and longitudinal views of shoot tips are used in the following discussion to show the spatial relationship of maturing vascular elements to the apical meristem and to the leaves of successive plastochrons. The relative ages of the leaves are expressed in plastochronic numbers assigned to the leaves in counting them from the apex downward, including the smallest structure definitely discernible as a leaf. The plastochron in which the first mature sieve element matures varies in relation to the state of activity of the apex, that is, whether or not it is producing a new primordium. To illustrate the plastochronic sequence of sieve-element differentiation, shoots with active apical meristems, that is, those with a recently formed primordium (fig. 2), are used.

Figure 2, *B*, illustrates the arrangement of successively older leaves around the axis of the shoot and figure 3 represents the vascular bundles of leaves of successive plastochrons at higher magnification. Leaves 2 (fig. 3, *A*) and 5 (fig. 3, *D*) are from the shoot illustrated in figure 2, *B*. For the purpose of better photography, leaves 3 (fig. 3, *B*) and 4 (fig. 3, *C*) were selected from

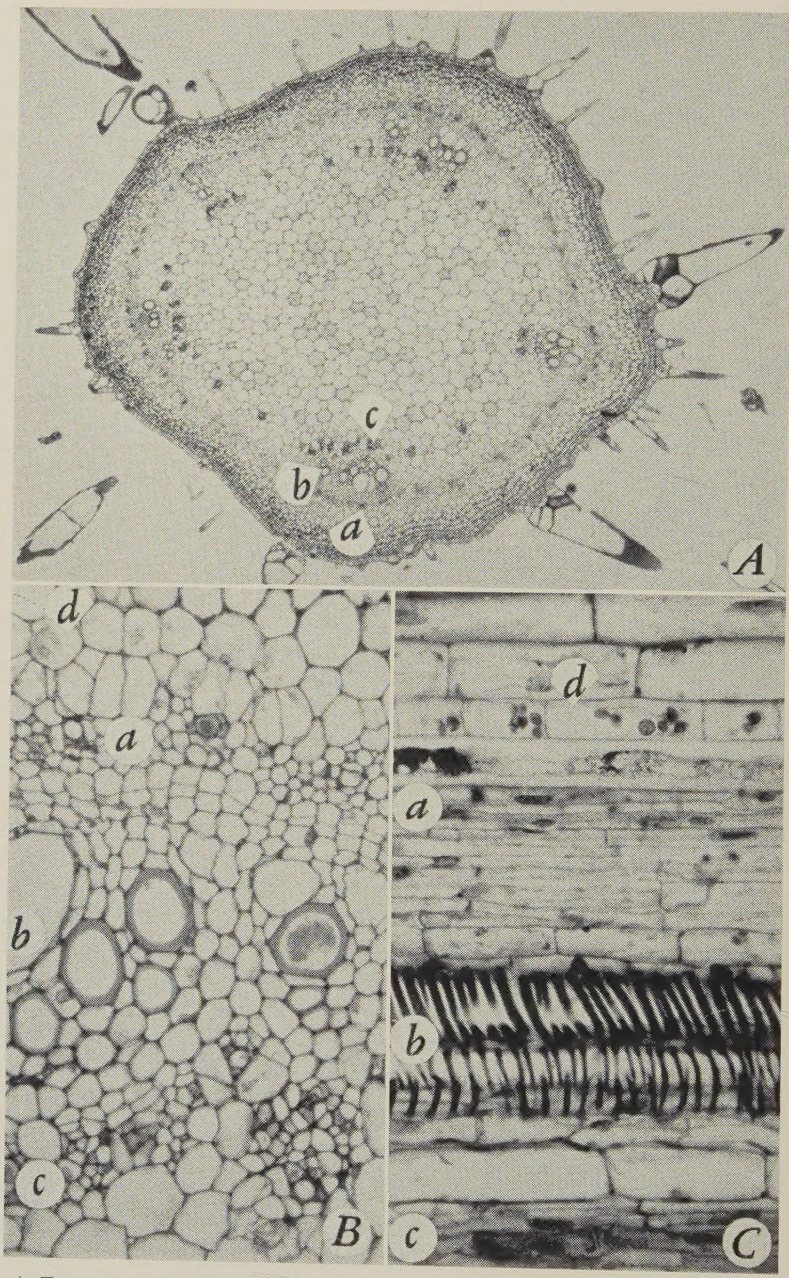

Fig. 4. Transverse (*A*, *B*) and longitudinal (*C*) sections of stem of a healthy tomato plant in primary state of growth. Details: *a*, external phloem; *b*, xylem; *c*, internal phloem; *d*, starch sheath. The enlarged cells beneath the starch sheath are fiber primordia. (*A*, × 47; *B*, *C*, × 330.)

other shoots. As mentioned previously, a primordium was present above leaf 1. Only its procambium is discernible in figure 2, *B*, at *c*. Leaf 1 (fig. 2, *B*) has a procambial strand without mature vascular elements. A bud primordium (*b*) appears in the axil of this leaf. Leaf 2 (fig. 3, *A*) has an immature sieve element (*a*) in its procambial strand. The protoplast of this element was still rather dense and had a nucleus. Leaf 3 (fig. 3, *B*) exhibits the first mature sieve element (*a*) of the external phloem in the median abaxial position of the procambial strand. Usually, as in figure 3, *B*, the first mature sieve element is rather narrow, but it stands out among the more densely staining adjacent cells because of its somewhat thick, darkly stained wall, and a clear lumen. The first mature external sieve element is usually associated with a companion cell (below and slightly to the left of the sieve element in fig. 3, *B*). No mature internal phloem and xylem were present in leaf 3.

In shoots with an inactive apical meristem (fig. 1) lacking a primordium, the first mature sieve element usually occurs in plastochron 1. However, whether the first sieve element occurs in plastochron 1 or 2, its maturation precedes that of the first protoxylem and internal phloem elements.

In the active shoot, the first mature protoxylem element was found in leaf 4 (fig. 3, *C*, at *b*). In the same leaf, at least one additional sieve element had differentiated in the external phloem (fig. 3, *C*, at *a*), and an immature sieve element was discernible in the internal phloem (fig. 3, *C*, at *c*). Leaves 3 to 5 (fig. 3, *B–D*) show that, whereas the first sieve element matures in median abaxial position, the succeeding ones usually differentiate right and left from the median position and appear successively closer to the margins of the vascular bundle. The sieve elements and associated cells occur in groups, and each group is separated from another by layers of parenchyma of varying width (Hayward, 1938). This intervening parenchyma has been called primary ray in tobacco (Esau, 1938). Eventually the sieve elements are obliterated in the order in which they appear.

The sieve elements of the internal phloem mature in rapid succession—or even several at the same time. Figure 3, *D*, shows some of the mature internal sieve elements in leaf 5. In another shoot tip, leaf 3 also had the first mature external sieve element, but leaf 4 had a mature protoxylem element as well as several mature sieve elements in the internal phloem. In the same shoot tip, the first external sieve element of leaf 4 was undergoing obliteration, while another sieve element of the external phloem had differentiated in the centripetal direction from the first. In other shoots the beginning of obliteration was observed in leaf 5 (e.g., fig. 3, *D*, at *d*). Thus, it appears that in tomato the first internal sieve elements mature at the time when the first external sieve elements are obliterated. Leaf 5 in figure 3, *D*, had 10 mature sieve elements in the external phloem, nine in the internal phloem, and three mature xylem elements. The obliteration of the first internal sieve element was found in leaf 6.

The internode below leaf 7 (fig. 4, *A*) clearly reveals the effect of primary growth of the shoot on the phloem. During the elongation of the stem the first sieve elements and accompanying companion cells (the protophloem) are completely obliterated. However, their original position may be identified

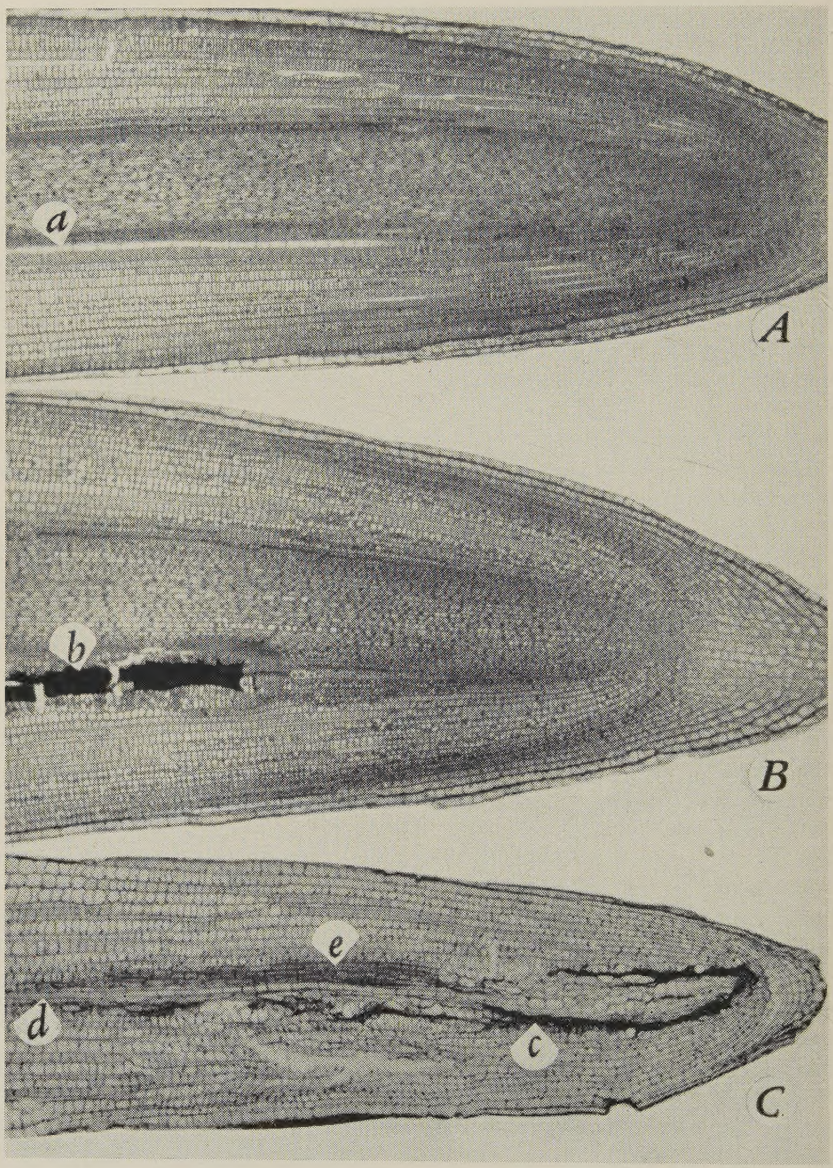

Fig. 5. Longitudinal sections of root tips from a healthy tomato plant (*A*) and from plants infected with curly top (*B*) and aster yellows (*C*). The apical meristem and the root cap are to the right in each view. Details: *a*, series of sieve elements composing the first sieve tube (protophloem); *b*, primary necrosis associated with the first sieve tube (not visible); *c*, secondary necrosis; *d*, series of xylem elements; *e*, hyperplastic phloem. (All × 70.)

by the crushed remnants among the fiber primordia that constitute part of the protophloem (fig. 4, *B*, large cells below the starch sheath, *d*). These fiber primordia are thin-walled, long, relatively wide, multinucleate cells with inclusions, probably tannins, that take up lacmoid stain rather strongly (fig. 4, *C*, part of long cell below starch sheath, *d*).

Centripetally from the fiber primordia is the metaphloem (fig. 3, *B*, at *a*), which is characterized by more numerous companion cells and parenchyma cells than the protophloem. The sieve elements are also somewhat wider and longer than those of the protophloem.

The internal phloem is also affected by the primary growth of the shoot. The first-formed sieve elements, which have been obliterated, appear as dark-stained streaks near the inner margin of each internal phloem group (fig. 4, *B*). As the earlier sieve elements and companion cells are obliterated, additional internal phloem elements are produced by division of cells within the group farther away from the center of the stem. The internal phloem groups are larger than those of the external phloem (fig. 4, *A*, *B*). No cambium is formed between the xylem and the internal phloem. Fiber primordia develop near the site of the crushed elements of the internal phloem. They are easily recognized when they begin to develop secondary walls.

The Sieve Element. The development of the sieve element in the Solanaceae has been thoroughly discussed for tobacco (Crafts, 1934; Esau, 1938) and potato (Artschwager, 1918; Crafts, 1933). The sieve element of tomato shows the same characteristics as those of tobacco and potato.

After the division of the sieve-element mother cell into a sieve element and a companion cell, one or two slime bodies differentiate in the sieve-element protoplast (fig. 16, *A*, *a*, p. 500). They may be spindle-shaped or more or less strongly twisted, resembling a corkscrew. When first formed, the slime body appears dense and stains deeply. At that time the nucleus still has its normal density, and its nucleolus is discernible (fig. 16, *A*, p. 500). Later the slime body assumes a loose, fibrous structure (fig. 16, *B*, *a*, p. 500) and finally disperses completely. Meanwhile the nucleus becomes vacuolated, and its nucleolus disappears (fig. 16, *B*, *b*, p. 500). The nucleus disintegrates as the slime bodies disperse. The contents of the sieve element assume the characteristic clear appearance, except for the accumulation of coagulated slime on the sieve plates in killed material. The sieve elements of tomato have plastids which stain only faintly with cytoplasmic stains, but which form the characteristic sieve-element carbohydrate that stains reddish-purple with iodine.

Primary-phloem Development in the Root. In the study of development of the first sieve elements, the roots are particularly useful because their primary-tissue organization is simpler and more regular than that in the shoot, partly because it is not complicated by connections with lateral appendages. The first sieve elements, with their clear contents, stand out among the meristematic cells and form continuous vertical rows (fig. 5, *A*). Because of this arrangement the stages of development of the sieve elements can be readily followed from near the apex to the more mature part of the root.

Like tobacco (Esau, 1941), tomato has three tiers of apical initials in the root. These are the initials of the central cylinder, the cortex, and the root

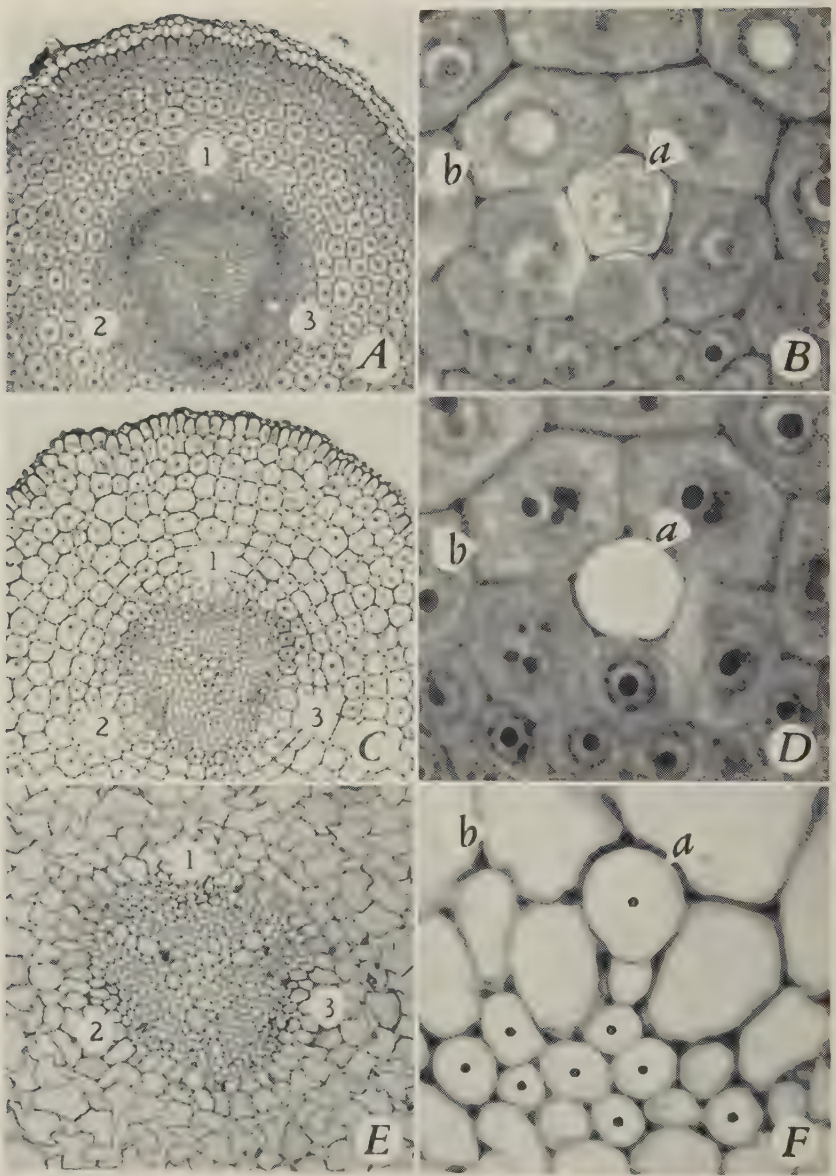

Fig. 6. Transverse sections of root tips from a healthy tomato plant taken successively farther from the apical meristem. The numbers 1–3 occur just outside the phloem poles. Mature protophloem elements occur in *A* (630 μ from the apex) and *C* (1,150 μ from the apex), one at each pole. Several sieve elements and several mature xylem elements (protoxylem and some metaxylem) are present in *E*. *B*, *D*, and *F* show phloem poles with immature sieve element (*B*), mature sieve element (*D*), and several mature sieve elements (dots in *F*). Details: *a*, protophloem sieve element; *b*, pericyclic cell; dots, sieve elements (all metaphloem, except *a*). (*A*, *C*, $\times$ 110; *E*, $\times$ 160; *B*, *D*, *F*, $\times$ 1,000.)

cap. By periclinal divisions, the root-cap cells give rise to the epidermis. The cortex consists of a simple, homogeneous parenchyma (fig. 6, *A*, *C*). The cortical cells are arranged in relatively orderly radial rows associated with conspicuous intercellular spaces. This orderly arrangement results from repeated periclinal divisions during the increase in the circumference of the cortex. These periclinal divisions begin in the recent derivatives of the cortical initials and are repeated in the inner of two derivatives from the successive periclinal divisions. After the periclinal divisions are completed, the innermost layer of the cortex develops into the endodermis. Sometimes an additional periclinal division occurs in the young endodermis opposite a phloem pole. If such a division takes place, only the inner cell differentiates as an endodermal cell, and its Casparian strip is continuous with that of the cells that did not divide. The Casparian strips are first observed approximately at the level where the first xylem elements mature.

The central cylinder is distinct from the cortex in that its cells are rather compactly arranged, whereas intercellular spaces are conspicuous in the cortex (fig. 6, *A*, *C*). The outermost layer of the central cylinder, the pericycle, becomes defined close to the apical meristem.

The future metaxylem becomes visible about 20 microns from the apex because of perceptible vacuolation and enlargement of its cells. The primordia of the metaxylem elements are arranged in rows radiating from the center of the root. At the outer ends of each row, protoxylem elements differentiate eventually. The number of radiating rows of the xylem indicates the number of xylem and phloem poles that are to develop. The number of these poles varies from two to four, that is, the root xylem may be diarch, triarch, or tetrarch. *A*, *C*, and *E*, of figure 6 are taken from a triarch root. The phloem poles are marked 1, 2, and 3. The first immature protophloem sieve element was evident at pole 2, at 220 microns, and at poles 1 and 3, at 230 microns from the apical initials. The first mature element was found at pole 1 at 450 microns; at pole 2, at 490 microns; and at pole 3, at 500 microns from the apical initials. Figure 6, *A*, taken 630 microns from the apex, depicts the level at which all the sieve elements were mature. Figure 6, *C*, taken at over 1,000 microns from the apical initials, shows the vacuolated condition of the future primary xylem. Only one sieve element was mature in each pole at this level.

The sieve element does not differ from the rest of the cells of the central cylinder in the procambial state. From that point it gradually elongates, the cytoplasm becomes less dense, and the walls thicken somewhat (fig. 6, *B*). In some roots, slime bodies were discerned in differentiating sieve elements. The nucleus and slime bodies stain pink with Giemsa stain, later disintegrate, and the sieve element becomes clear (fig. 6, *D*). At that time the sieve element appears highly turgid as judged by its rounded outline in cross section in contrast to the angular outline of younger cells (compare *B* and *D* in fig. 6). The nucleolus of the disintegrated nucleus may remain for a time in the lumen of a seemingly functional sieve element. Ultimately, the nucleolus, too, disintegrates. Callose was not observed in the mature protophloem sieve elements either by means of fluorescent-light technique (Currier and Strugger, 1956) or by the use of the staining combination developed for revealing

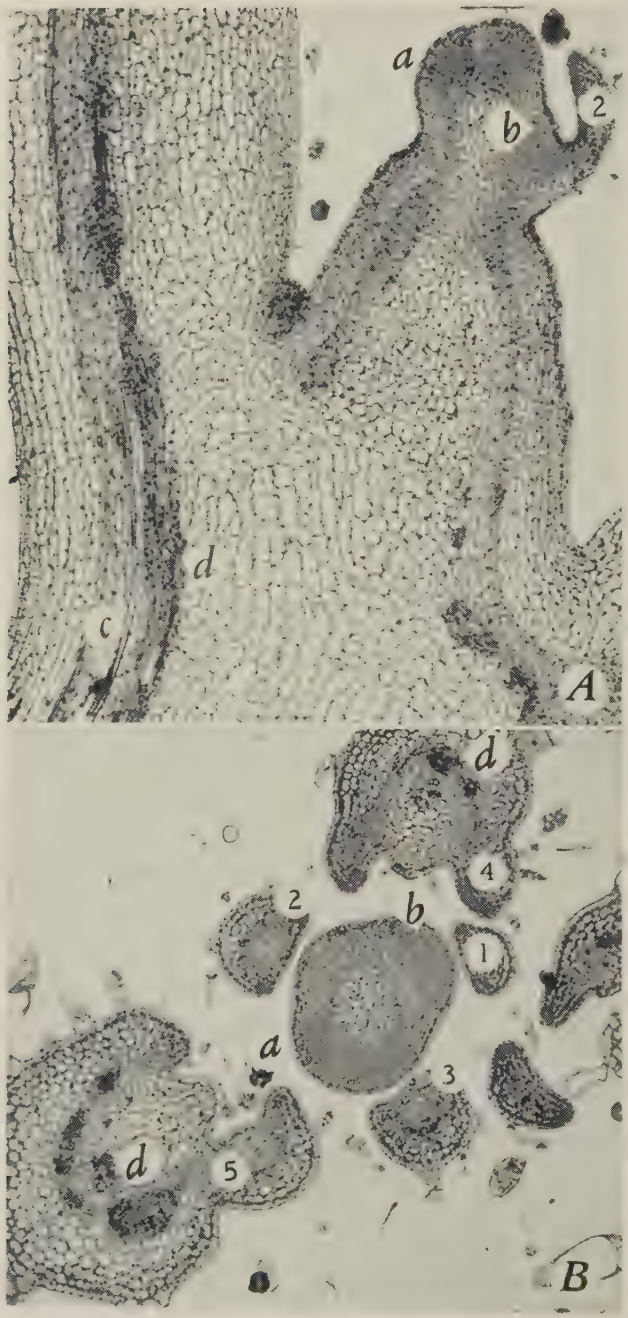

Fig. 7. Longitudinal (*A*) and transverse (*B*) views of curly top shoots of tomato, both with primordia (*a*). The numbers 1–5 indicate plastochronic ages of leaves. Hyperplastic condition of the phloem (*d*) is conspicuous in the axillant leaf in *A* (to the left), in the external phloem of leaf 4 in *B*, and in both the external and the internal phloem in leaf 5 in *B*. Details: *a*, primordium on apical meristem; *b*, axillary bud; *c*, xylem; *d*, hyperplastic phloem. (Both × 85.)

callose in fixed material (Cheadle *et al.*, 1953). Esau (1941) saw nacreous walls in protophloem sieve tubes in tobacco root. Such walls were not observed in tomato.

The first protoxylem elements mature about 940 to 1,800 microns from the apex. The level of differentiation of the second sieve element and protoxylem element at each pole was not determined. According to Esau (1941), in tobacco the second protoxylem and second sieve elements differentiate about three times the distance of the first sieve element from the apex. If the same relation prevails in tomato, the second protoxylem element and the second sieve element would be mature at about 1,500 microns from the apex. In figure 6, *E*, four or five xylem elements are mature in each pole. The section for this plate was taken about 3 cm from the apex. An enlargement (fig. 6, *F*) of one of the phloem poles from this level shows nine sieve elements, with the first still intact (cell at *a*). This element is the only one that should be classified as a member of the protophloem tissue. The other, later, narrow elements are in the metaphloem.

PLANT AFFECTED BY CURLY TOP

External Symptoms. The first symptoms seen in curly-top plants inoculated by grafting was vein clearing of the leaves that arose from the lateral buds during the second week after grafting. Later these leaves became curly and the veins turned purple. (Occasionally purple veins were also observed in the normal plant.) Severin (1929) found, in tomato, white excrescences that resembled the wart-like protuberances on beets infected with curly top. Such protuberances were not observed in the present study. Severin's article has excellent illustrations of the external symptoms of curly top on tomato.

The original plant, infected by means of leafhoppers, from which the scions were collected for grafting was severely stunted and yellow in contrast to the control plants. The grafted plants did not develop pronounced stunting and yellowing until kept in the greenhouse for about a month after the collection period. In such plants mature leaves became brittle. This symptom was followed by a general yellowing, and finally death of the plants. Some grafted plants recovered for a while, but later died. As mentioned under "Material and Methods," it was difficult to maintain the virus culture because of the death of the grafted plants; successive grafts had to be made.

Phloem Degeneration in the Shoot. Like other hosts of the curly top virus (cf. Esau, 1935*b*, 1941; Girolami, 1955), tomato infected with curly top showed, in the shoot, stages of early differentiation and procambialization fundamentally similar to those in the normal plant. Moreover, the meristematic activity at the shoot apex was not noticeably affected by the presence of the virus, at least during the collection period. Esau (1941) found that curly top virus decreased the growth of the tobacco plant. It should be noted, however, that Esau employed young seedlings and infective leafhoppers to obtain curly top-diseased material. In the present study the tomato plants used for inoculation by grafting were vigorously growing, two-week-old specimens. Possibly the period between inoculation and collection was not long enough for the virus to have affected the growth of the relatively large plants. Eventually, however, these plants became yellow and died.

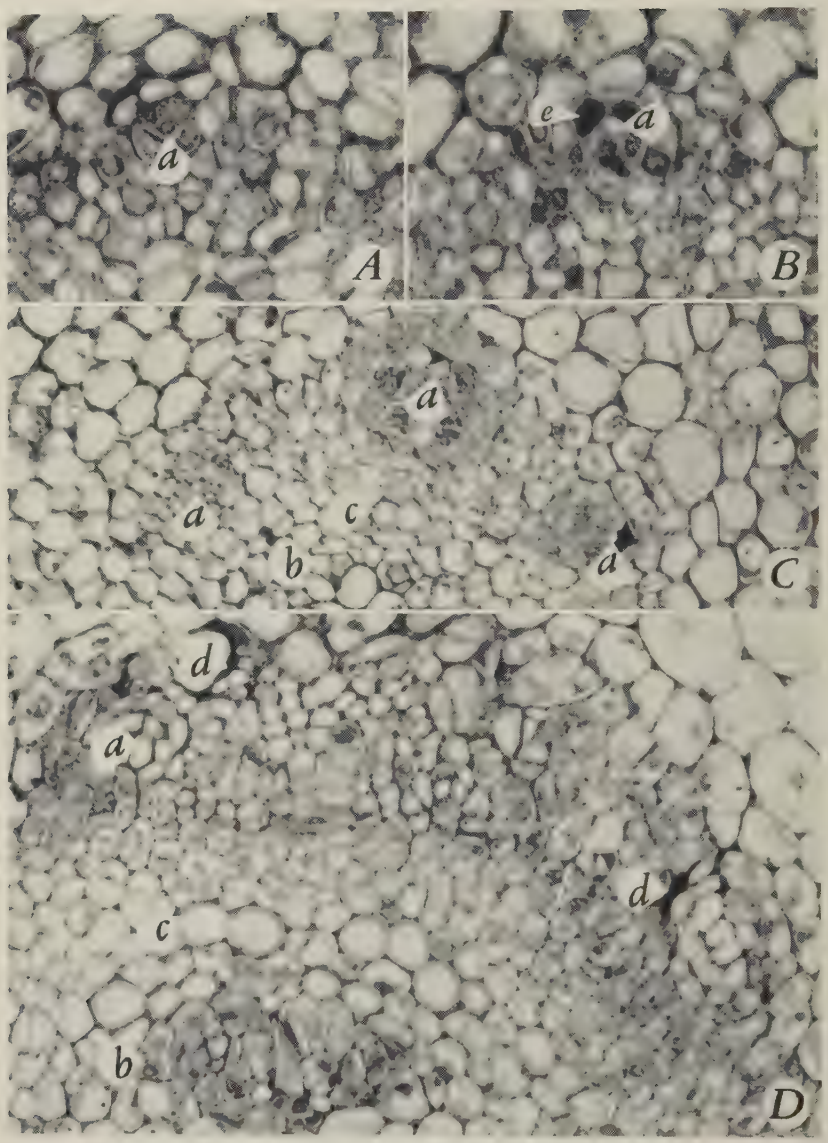

Fig. 8. Transverse views of vascular tissues in successively older leaves from a curly top tomato plant. Leaves 2–4 (*A–C*) were taken from the same shoot as figure 7, *B*; leaf 5 was from another shoot. In *A*, the first sieve element (*a*) is immature; in *B*, it is mature and one of its neighboring cells (*e*) shows primary chromophily and slight hypertrophy; in *C*, some primary hyperplasia and primary necrosis are evident next to two sieve elements (above and to the right); in *D* the hyperplastic phloem shows clearing, and some secondary necrosis has occurred (*d*). Details: *a*, sieve element; *b*, internal phloem; *c*, xylem; *d*, necrosis; *e*, chromophilic cell. (*A*, *B*, × 780; *C*, *D*, × 490.)

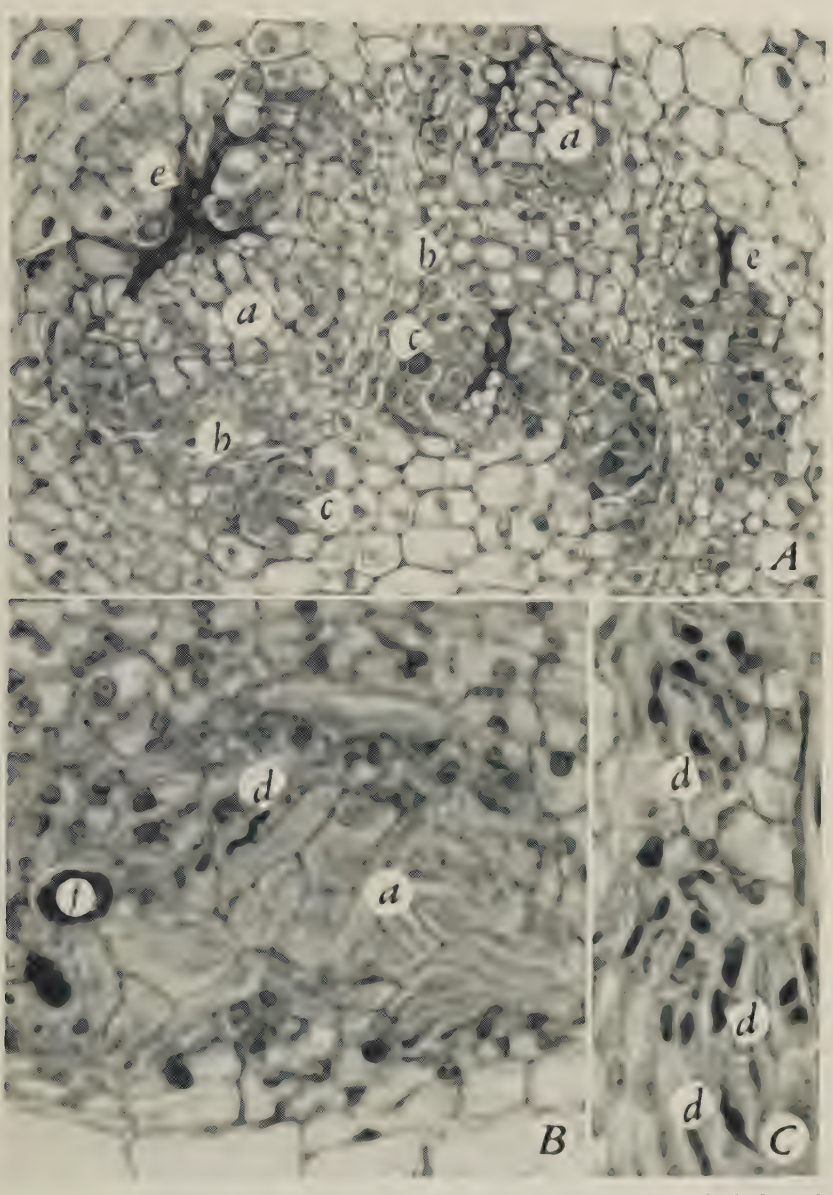

Fig. 9. *A*, transverse view of vascular tissues from curly top tomato leaf of plastochron 6, with cleared hyperplastic phloem (*a*) undergoing secondary necrosis (*e*). At *c*, in median position, the hyperplastic internal phloem has disrupted the xylem. *B*, *C*, longitudinal views of hyperplastic phloem from curly top stem, showing sieve bodies (*d*) and cleared cells (*a*). (All × 490.)

The sequence of phloem degeneration was followed in leaves of successive plastochronic ages, as was that of the normal development of the tissue. The maturation of the first vascular elements occurs in the same sequence in diseased shoots as in the healthy; at first, some sieve elements mature in the external phloem, then follow some xylem elements, and finally sieve elements of the internal phloem. Soon after the first sieve elements mature, however, degenerative changes begin to appear in the phloem, and the later development of that tissue is much modified. The sequence of these events is described in the following paragraphs by the use of shoots similar to the active, healthy ones depicted in figures 2 and 3.

Figure 7, *A*, illustrates a lateral shoot, attached to the parent shoot, from a diseased plant. Its apical region resembles that of the healthy plant in figure 2, *A*. The subapical region, where only procambium is visible in the vascular region, is also normal. Symptoms of the disease are evident only at levels where mature phloem is present. This tissue appears more massive than normal because of extensive hyperplasia (e.g., fig. 7, *A*, *B*, at *d*).

The sequence of the degeneration is best followed by the use of cross sections. Figure 7, *B*, a section 140 microns below the shoot apex, shows leaves of plastochronic ages similar to those of the leaves in the active, normal shoot in figure 2, *B*. Leaves 1 to 4, figures 7, *B*, and 8, *A* to *C*, show the stages of phloem degeneration. Leaves 5 (fig. 8, *D*) and 6 (fig. 9, *A*) were selected from another shoot to show the so-called clearing and necrosis of the hyperplastic tissue.

The active state of the shoot tip in figure 7, *B*, is indicated by the presence of a rather large primordium (*a*). No degeneration was observed above this primordium, that is, in the apical meristem, nor below it, where procambium was differentiating in the axis and in leaves of plastochrons 1 and 2 (figs. 7, *B*, and 8, *A*).

In the shoot in figure 7, *B*, the first mature sieve element is found in leaf 3 in the median abaxial position of the vascular bundle (fig. 8, *B*, at *a*). One cell next to this sieve element shows the first evidence of degeneration: it is somewhat hypertrophied and chromophilic (*e*). In this leaf, hyperplasia is not evident. The finding of the initial anatomical effect in a leaf with a mature sieve element confirms the previous findings that the vertical spread of the effects of curly top virus depends on the presence of differentiated sieve elements (e.g., Esau, 1941, 1957). The first sieve element in leaf 3 appears to have matured normally; as mentioned, only one of the neighboring cells became affected. No differentiated internal phloem and protoxylem are visible in leaf 3.

In leaf 4 (fig. 8, *C*) the first apparently normal sieve element in median abaxial position of the external phloem is still intact, and two others have differentiated to the left and to the right of the first (*a*). At levels other than that of figure 8, *C*, the sieve element in the right phloem strand was not so clear as normal, mature sieve elements usually are. It contained some stained material other than slime although the nucleus was absent. The element was possibly partly degenerated. The immediate neighboring cells of the sieve elements in the center and to the right have undergone hyperplasia, and some cells are necrosed (appear black). These phenomena are

the primary hyperplasia and primary necrosis as defined by Esau (1935*a*). The necrosed cells have been crushed. The hyperplastic tissue is still meristematic; its cells are closely packed, and have dense cytoplasm. The sieve element and neighboring cells to the left are seemingly normal. The immature internal phloem (*b*) shows no degeneration. Two protoxylem elements are mature, and no abnormalities are associated with them.

Leaf 5 in figure 8, *D*, illustrates the maturation of the hyperplastic tissue. Cells that have undergone primary necrosis (*d*) appear as darkly stained areas. They are flanked centripetally by rather clear cells (*a*) of the mature hyperplastic tissue. This clear tissue is surrounded by immature hyperplastic tissue with contents that are still dense and chromophilic. The internal phloem (*b*) has mature sieve elements and is hyperplastic.

Leaf 6 (fig. 9, *A*), taken from the same shoot as leaf 5, shows secondary necrosis, that is, death and collapse of the cleared hyperplastic tissue (*c*). The tissue collapses in large masses, but some cells in the neighborhood undergo secondary hypertrophy and hyperplasia, and occupy the space left by the collapsed cells. Therefore a lacuna is not formed. Lacunas resulting from a collapse of hyperplastic tissue were observed in the sugar beet root (Esau, 1935*b*), tobacco shoot (Esau, 1941), and flax shoot (Girolami, 1955), all infected with curly top. In tomato the necrosed tissue is compressed by the greatly enlarged and divided cells (fig. 9, *A*, near *c*). Eventually all the primary rays and parenchyma of the xylem become hyperplastic. The cambium loses its identity completely and is replaced by hyperplastic tissue. Cortical cells located near the phloem also undergo hyperplasia.

The leaf in figure 8, *D*, and the stem sections in figure 10 show contrasts in the localization of degeneration in the phloem. In figure 8, *D*, the external phloem shows a more severe hyperplasia than the internal phloem. Figure 10 shows the reverse situation. Here the internal phloem is severely degenerated, whereas the external shows abnormalities only in relatively small areas. Esau (1935*b*) found a similar uneven distribution of hyperplasia in curly top sugar beets: the growth layers in the root that were differentiated before the onset of virus infection did not develop phloem degeneration. As noted earlier, in the normal tomato shoot, the external phloem precedes the internal in maturation of sieve elements. Apparently the leaf in figure 8, *D*, was relatively young, and the external phloem was not fully differentiated when the virus infection took place. Therefore, the external phloem became severely deranged. In contrast, the stem in figure 10 must have been relatively mature, and the external phloem advanced in differentiation before the virus entered that part of the plant. Therefore, the younger, internal phloem became more severely deranged than the older, external.

The hyperplastic tissue requires special attention because some of the earlier workers on curly top-diseased plants (e.g., Esau, 1935*a*, *b*, 1941; Girolami, 1955) identified this tissue as abnormal phloem composed mostly of more or less misshapen sieve elements, whereas others (Artschwager and Starrett, 1936) questioned the similarity between sieve elements and the hyperplastic cells.

As mentioned previously, the hyperplastic tissue is at first highly chromophilic, but as it matures the cells become clear—as clear, in fact, as the

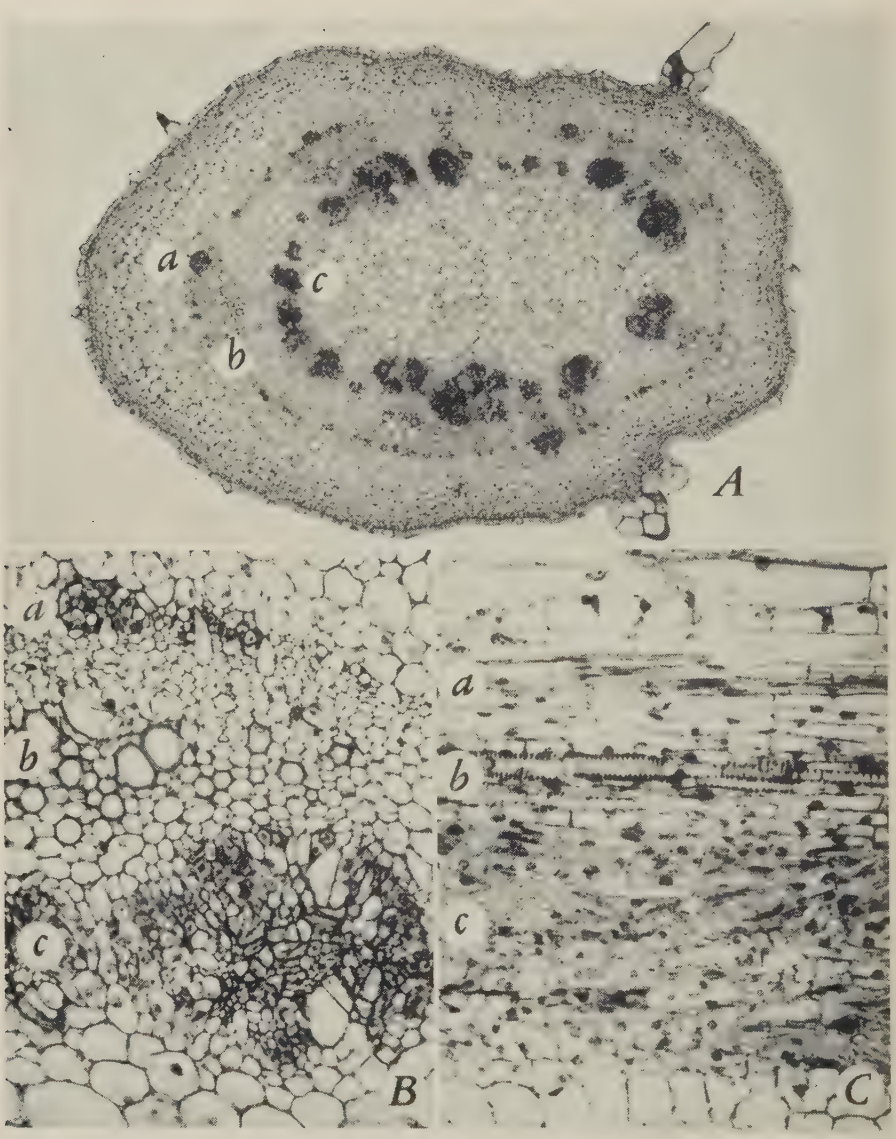

Fig. 10. Transverse (*A*, *B*) and longitudinal (*C*) sections of stem of curly top tomato plant in primary state of growth. The internal phloem (*c*) shows more severe hyperplasia than the external phloem (*a*). Details: *a*, external phloem; *b*, xylem; *c*, internal phloem. (*A*, × 42; *B*, *C*, × 200.)

mature sieve elements. The stages in the development of clear cells are best seen in longitudinal sections (figs. 9, *B*, *C*, and 10, *C*). In such views, rather long, clear, apparently normal sieve elements may be found surrounded by hyperplastic cells of various sizes and forms, frequently relatively short. The hyperplastic cells are irregularly arranged, and appear in various stages of development from densely cytoplasmic to entirely clear. In the intermediate stages, slime bodies may be seen in many of these cells (fig. 9, *B*, *C*, at *d*). These show the same form as the slime bodies in the normal sieve elements described previously (fig. 16, *A*, *B*, p. 500). Cells with slime bodies contain normal nuclei. Both nuclei and slime bodies stain pink with Giemsa stain. Eventually they break down, and the cytoplasm ceases to be chromophilic. Thus, the hyperplastic cells pass through cytologic changes similar to those previously described for the developing normal sieve elements, except that the cytoplasm in the young hyperplastic cells is usually abnormally chromophilic, and the nuclei may be hypertrophied. In sections stained with resorcin blue, callose may be seen in the cleared cells. Because of the large numbers of abnormal sieve elements in the hyperplastic tissue, more callose is found in a given section of diseased tissue than in the normal. Moreover, the callose in the lateral sieve areas is more prominent than in the normal sieve elements. Thus, the clear hyperplastic cells show at least some of the typical characteristics of a sieve element and can be designated as abnormal sieve elements. Companion cells have not been found in connection with these sieve elements in curly top tomato. Occasional ones were found by Esau (1941) in tobacco. Girolami (1955) did not find any companion cells in curly top flax. Figure 9, *B* and *C*, illustrates the hyperplastic cells in various stages of development. In figure 9, *B*, some sieve elements contain nuclei, and one shows a dense slime body. Others are devoid of discrete nuclei and are rather clear. A dense hypertrophied cell appears at *f*; its wall was lined with callose. In figure 9, *C*, many cells contain nuclei and slime bodies. In normal phloem, sieve elements with slime bodies are encountered only a few at a time. Moreover, the maturation of the hyperplastic tissue is not so orderly as that of the normal phloem: various stages of differentiation are intermingled. As mentioned previously, the clear cells eventually die and collapse (secondary necrosis), and are replaced by proliferating parenchyma cells (wound-healing reaction, or secondary hyperplasia).

Phloem Degeneration in the Root. The stages of phloem degeneration are more easily traced in the root than in the shoot because, as pointed out previously, the initial sieve tubes in the root form no anastomoses, and extend in a straight line for long distances; moreover, they are clearly distinguishable cytologically from the neighboring cells. Figure 5, *A*, illustrates the continuity of the sieve tube and figure 5, *B*, the close association with the sieve tube of the degeneration induced by curly top. The stages of degeneration are shown by means of sections taken from different levels of two root tips (fig. 11, *A*, *B*, *C*). In addition, figures 11, *D*, *E*, and 12 illustrate, in cross sections of the phloem poles taken at relatively high magnification, certain details of degeneration.

The roots collected during the second week after grafting showed no

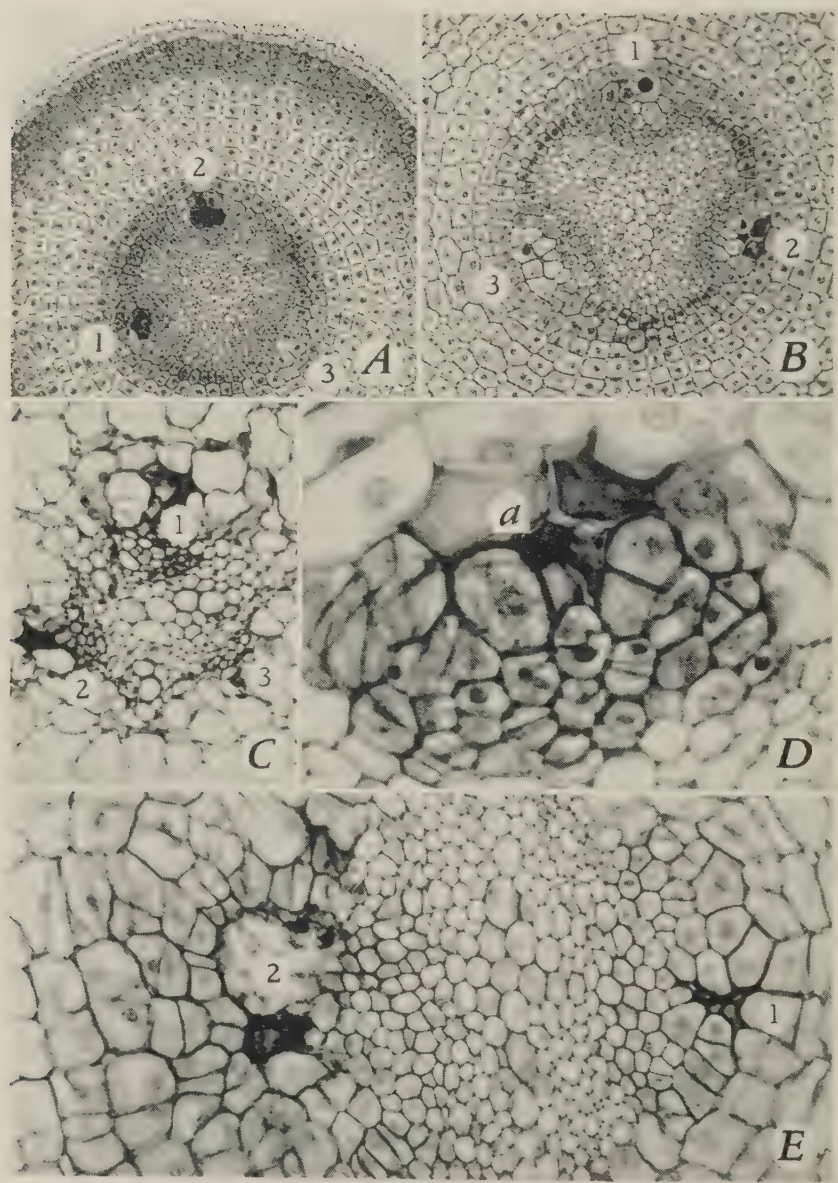

Fig. 11. Transverse sections of roots of curly top tomato plants. The sections *A–C* were taken 450 μ, 600 μ, and 1.5 cm from the apex. The numbers 1–3 indicate the phloem poles. *D*, hyperplastic phloem associated with gum-occluded protophloem sieve element (*a*). *E*, diarch root with secondary necrosis at both poles and a lacuna at pole 2. Considerable secondary hyperplasia in the cortex at pole 2. (*A–C*, × 110; *D*, × 1,000; *E*, × 250.)

symptoms. The fourth-week collection showed varying degrees of degeneration. Some roots were less affected than others, and some were free of symptoms. In roots that showed less pronounced degeneration, the meristematic activity of the apical meristem was not disturbed, and the sieve element matured at about the same level as in the normal root (compare *A* and *B* in figure 5). This type of root was found to be most useful for the detailed study of the cytoplasmic changes in the phloem. In roots with severe symptoms, the activity of the apical meristem was depressed, and the primary vascular tissues matured closer to the apex than in normal roots. A very striking characteristic of these roots was their great thickness which, histologically, was expressed in a larger-than-normal number of cells in the cortex and central cylinder as seen in transections. Such roots gave the appearance of abnormally stimulated meristematic activity close to the apical meristem.

Although the curly top virus produces a pronounced degeneration in the phloem, the apical meristem does not appear to be deranged except that it may become inactive. Some affected roots, for example, showed a high degree of vacuolation of their cells even in the apical meristem. Such roots took up less stain than normal roots. Even the less severely affected roots tended to stain more lightly than normal. In the relatively highly vacuolated root tips, the Casparian strips were observed as soon as the endodermis was formed. This early maturation of the endodermis, like the maturation of xylem close to the apex, was related to the cessation of meristematic activity. In the diseased roots, however, the Casparian strips appeared much closer to the apex than did the xylem, whereas in the normal root the endodermis and xylem matured at approximately the same level. The diseased roots behaved somewhat like dormant roots of certain plants in which suberization occurs in the apical region; in fact, in dormant roots the apical meristem may be enclosed by a suberized sheath (cf. Wilcox, 1954).

The two roots from which the sections for figure 11, *A*, *B*, and *C* (*A* and *B* from one root; *C*, from another) were taken showed relatively mild degeneration, so that the sieve tubes differentiated at about the same distance from the apex as in the normal root. The three sieve tubes marked 1, 2, and 3 became mature at somewhat different distances from the apex, again a feature common in normal roots. As in the shoot, the apical meristem in the root was free from any visible degeneration. The first symptoms were found at phloem poles 1 and 2 of the root shown in figure 11, *A* and *B*, in the following sequence. About 200 microns from the apex the three sieve elements were beginning to show evidence of differentiation like that in the normal root. Their cytoplasm became less dense and their walls thickened, although their nuclei were still present. Degeneration was not found at this level. About 320 microns from the apex, sieve elements 1 and 2 both contained gum,[6] but their nuclei were still present (fig. 12, *A*; nucleus was discernible under the microscope). The neighboring cells were seemingly normal, a condition that existed for some distance into the more mature levels of the root. Sieve element 3 appeared to have matured more or less normally at 320 microns from the apex. Its nucleus disappeared and its contents became clear except for a

[6] "Gum" is used here in a wide sense to indicate the presence of deeply staining products of cell breakdown.

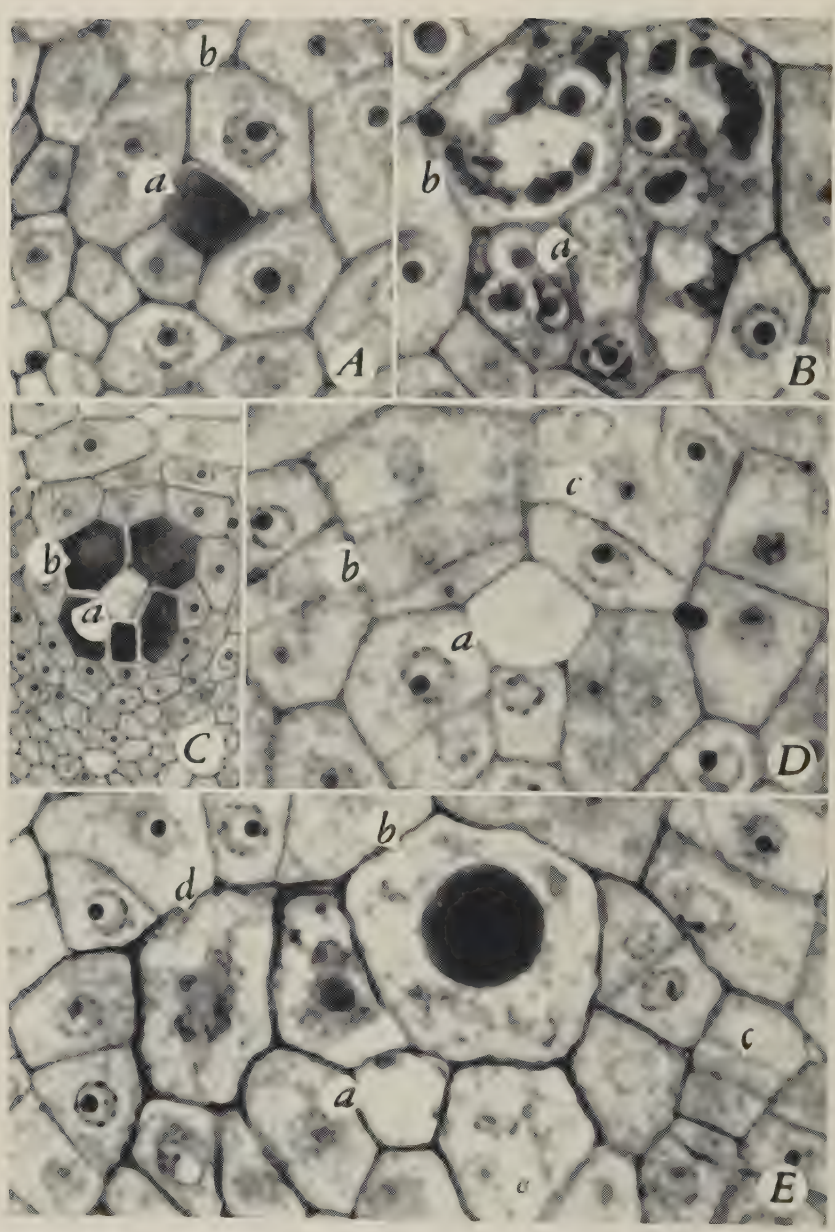

Fig. 12. Transverse sections of roots of curly top tomato plants. Each shows one phloem pole with various patterns of degeneration. *A*, sieve element (*a*) occluded by gum. Cells surrounding the sieve element (*a*) contain bodies in *B*, are highly chromophilic in *C*, have undergone hyperplastic divisions in *D*, and show cytoplasmic network in *E*. Details: *a*, sieve element; *b*, pericycle cell (with a dense nucleus in *E*); *c*, cell wall from recent hyperplastic division; *d*, cell with network and degenerating nucleus; *e*, intact nucleus in cell with network. (*A*, *B*, *D*, × 1,000; *C*, × 440; *E*, × 820.)

small amount of some stained material other than slime, possibly degenerated cytoplasm. Its neighboring cells appeared normal at this level.

At the 450-micron level (fig. 11, *A*), where normally the sieve elements are mature, sieve element 2 was still plugged, but the nucleus was no longer visible. Here, the neighboring cells also were chromophilic and contained cytoplasmic bodies of various shapes and sizes similar to those shown in figure 12, *B*. Hypertrophy and hyperplasia were not observed among these chromophilic cells. In another root, at about the same level as shown in figure 11, *A*, one sieve element had only lightly stained contents but the neighboring cells were highly chromophilic (fig. 12, *C*). When stained with tannic acid-ferric chloride-resorcin blue, the chromophilic cells sometimes showed very bright blue, sometimes almost black. The protoplasts of some of the chromophilic cells became necrosed (primary necrosis).

About 510 microns from the apex, sieve tube 1 was still plugged and the neighboring cells were chromophilic like those adjacent to sieve tube 2. Cells at pole 3 were still unaffected. At this level, sieve tube 1 and its neighboring cells contained granular material instead of the darkly stained, homogeneous cytoplasm seen at the 450-micron level. This granular material disappeared at the 600-micron level (fig. 11, *B*), replaced by a chromatic network which filled the sieve element and neighboring cells (fig. 12, *E*). Artschwager and Starrett (1936) proposed that the chromatic network which they found in curly top-affected sugar beets was cytoplasmic and nuclear in origin. The present study suggests that the chromatic network is mainly cytoplasmic in origin because this network and the nuclei are evident in the same cells. Sometimes the nuclei are nearly normal in appearance, sometimes they are highly vacuolated, sometimes extremely dense (fig. 12, *E*, cell *b*; through the microscope, a nucleolus was discernible in this nucleus). The nuclei of the two pericyclic cells to the left of cell *b*, which also contain the chromatic network, are disintegrating. Thus the chromatic network may be present before and after nuclear breakdown. Therefore, it appears to originate in the cytoplasm, but possibly when the nucleus disintegrates it also contributes to the network. In normal differentiating sieve elements the cytoplasm becomes more or less stringy before clearing occurs. Possibly the chromatic network that forms in the sieve element and neighboring cells of curly top-diseased roots represents a similar modification of the protoplast such as occurs in the normal sieve elements during the dispersal of slime. The phenomenon is, however, more conspicuous in the diseased material because the network is often more chromophilic, and more cells develop such a network.

At the 600-micron level (fig. 11, *B*) some cells of the pericycle and endodermis at phloem poles 1, 2, and 3 were dividing (primary hyperplasia). With reference to sieve tube 3, primary hyperplasia was the first evidence of degeneration at this pole (fig. 12, *D*). At the 600-micron level (fig. 11, *B*) the sieve tube at pole 3 was crushed and the adjacent cells enlarged and lightly stained.

The chromatic network in the sieve element and neighboring cells eventually disappears, the cells become clear, the nucleus vacuolates, loses turgidity, and disintegrates completely. Primary necrosis now becomes more pronounced. It may involve the apparently normal sieve element, which may

collapse alone or together with its neighbors. The primary necrotic area sometimes stains bright blue like callose, at other times charcoal black; when safranin is used, the area may be bright red. In severe degeneration the sieve element and its neighbors may collapse (primary necrosis) without undergoing the cellular changes described above. An extensive hyperplasia follows such a collapse (fig. 11, *E*, pole 1).

When hyperplasia is severe it may spread in the centripetal direction from the first sieve element beyond the pericycle, that is, into the cortex (figs. 11, *E*, especially at pole 2; 19, *C*). The hyperplastic cells may or may not resemble the abnormal sieve elements described previously for the shoot. The hyperplastic cells originating in the pericycle and phloem commonly develop rather thick walls and slime bodies, and their cytoplasm decreases in density (fig. 11, *D*). The nucleus and slime bodies of these hyperplastic cells stain pink with Giemsa stain. Later the cytoplasm and slime bodies disintegrate but the cells do not clear so completely as do normal ones. A stringy material commonly remains in their lumina. Some hyperplastic cells do not form slime bodies, but become as clear as those that do. Such cells merely lose their nuclei, and their cytoplasm ceases to take up the stain. Both kinds of cells may be regarded as abnormal sieve elements because they have some features in common with normal sieve elements. Callose is found on the sieve areas of abnormal sieve elements. In longitudinal view the hyperplastic tissue shows that it is composed of short, haphazardly arranged cells.

The hyperplastic tissue is obliterated in large masses (secondary necrosis). Such necrosis is shown in figure 11, *C*, taken from a section about 1.5 cm from the apex of a root. Four or five xylem elements are mature in each xylem pole at this level. Sometimes the hyperplastic tissue is obliterated before the abnormal sieve elements become clear. When this happens, a black-stained mass is produced. Contrary to the situation in the shoot, the walls of the hyperplastic tissue in the root may disintegrate so that a lacuna is formed (fig. 11, *E*, pole 2). Commonly, however, the neighboring cells of the collapsed tissue hypertrophy, divide, and intrude into the space formed during secondary necrosis (fig. 19, *C*) thereby preventing the formation of a lacuna.

PLANT AFFECTED BY ASTER YELLOWS

External Symptoms. The plant that was inoculated by means of leafhoppers and served as the original source of the virus for the grafts was stunted, and showed a general yellowing. Its newly formed parts were thin and small.

The plants to which aster yellows shoots were grafted produced numerous secondary shoots. Some of the axillary buds which would normally remain dormant in healthy plants grew in these plants. This symptom has not been previously observed in tomatoes infected with Western strains of aster yellows and may have been associated with the removal of the terminal bud before grafting. Numerous adventitious roots also developed from the stem. No adventitious roots developed in the healthy and curly top plants during the collection period. In this study the aster yellows plants lived longer than the curly top plants. Some showed severe symp-

toms in the late fall and almost throughout the winter, but recovered during the following spring. The new shoots, however, showed symptoms of the disease. As mentioned before, some curly top-affected plants also recovered from the severe infection, but most plants affected with this disease died.

Phloem Degeneration in the Shoot. Retardation of apical meristematic activity was more pronounced in plants inoculated with aster yellows than in curly top plants. In sections, the apical meristems of aster yellows plants resembled those of normal, inactive shoots in that they had no primordia. In contrast to the healthy, inactive shoots, however, the aster yellows shoots had a relatively low apical dome which ranged from 20 to 40 microns in height. Despite the relative inactivity, the apical meristem and subjacent region in aster yellows plants appeared to be free of degenerative changes (fig. 13).

Leaves of comparable plastochrons were more mature in aster yellows plants than in either curly top-diseased or normal shoots. It is notable also that lateral buds, which continued to develop in curly top and normal shoots, ceased to appear in aster yellows shoots (compare figs. 2, 7, and 13). This observation seems to contradict the statement made before that growth of axillary shoots was stimulated in aster yellows plants. Those shoots, however, developed from buds that were initiated before the disease became established.

Figure 13, *A*, showing a longitudinal section of a shoot of an aster yellows plant, illustrates the low apical dome. The leaf to the right was identified as leaf 1, the base to the left pertains to leaf 2. Procambium is evident close to the apical meristem.

Figure 13, *B*, is a cross section 70 microns below the apex of a shoot similar to that in figure 13, *A*. The vascular bundles of leaves 2 to 5 of the shoot in figure 13, *B*, are shown at higher magnification in figure 14 to illustrate consecutively the stages of degeneration in the phloem. Leaf 7 (fig. 14, *E*), which belongs to the same shoot as leaves 2 to 5, but is not shown in figure 13, *B*, is used to show a late stage of degeneration.

Leaf 1 (fig. 13, *B*; not shown in detail) had, in the median abaxial position of the external phloem, an immature sieve element which was flanked by seemingly normal cells. No protoxylem and internal phloem were visible. Leaf 2 (fig. 14, *A*) had the first mature sieve element in the median abaxial position of the external phloem. This element was surrounded by relatively small chromophilic cells. Hypertrophied cells (primary hypertrophy), such as often occur in curly top-affected leaves of similar age, were not found in early stages of degeneration in aster yellows leaves. There were no mature elements in the xylem and no internal phloem in leaf 2.

In leaf 3 (fig. 14, *C*) the first sieve element in median abaxial position of the external phloem was flanked by a second element (below *a*). The first element was completely collapsed two sections below the level shown in figure 14, *C*. The cells centripetally from the first sieve element (cells at *d* and at levels below the section depicted) were hyperplastic and contained hypertrophied nuclei, which almost filled the lumina of the cells as seen in transections. The hypertrophied nuclei took up the stain rather strongly. The

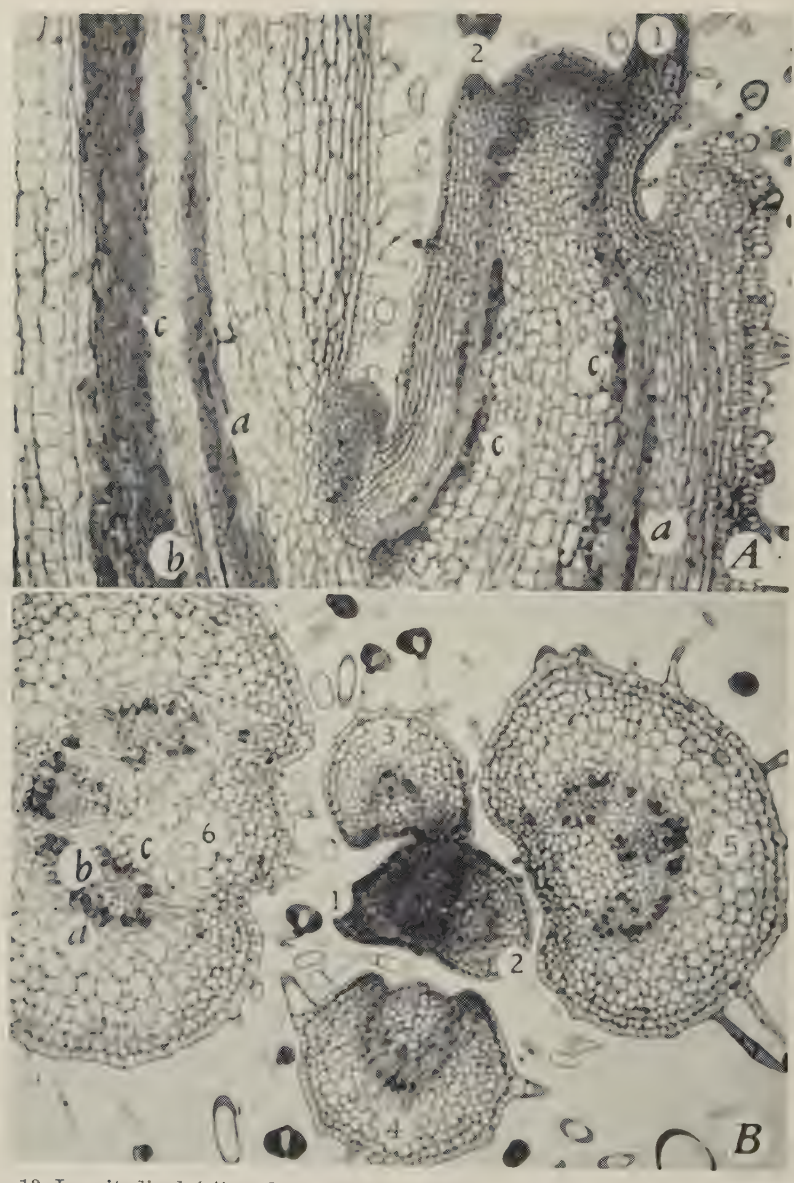

Fig. 13. Longitudinal (*A*) and transverse (*B*) views of aster yellows shoots of tomato. The nipulars 1–6 initiate plastochronic ages of leaves. Hyperplasia is evident in external (*a*) and internal (*c*) phloem of axillant leaf (to the left) and axillary shoot in *A*, and in leaves 5 and 6 in *B*. Details: *a*, external phloem; *b*, xylem; *c*, internal phloem. (Note *a* and *c* are reversed in axillant leaf in *A*.) (Both × 85).

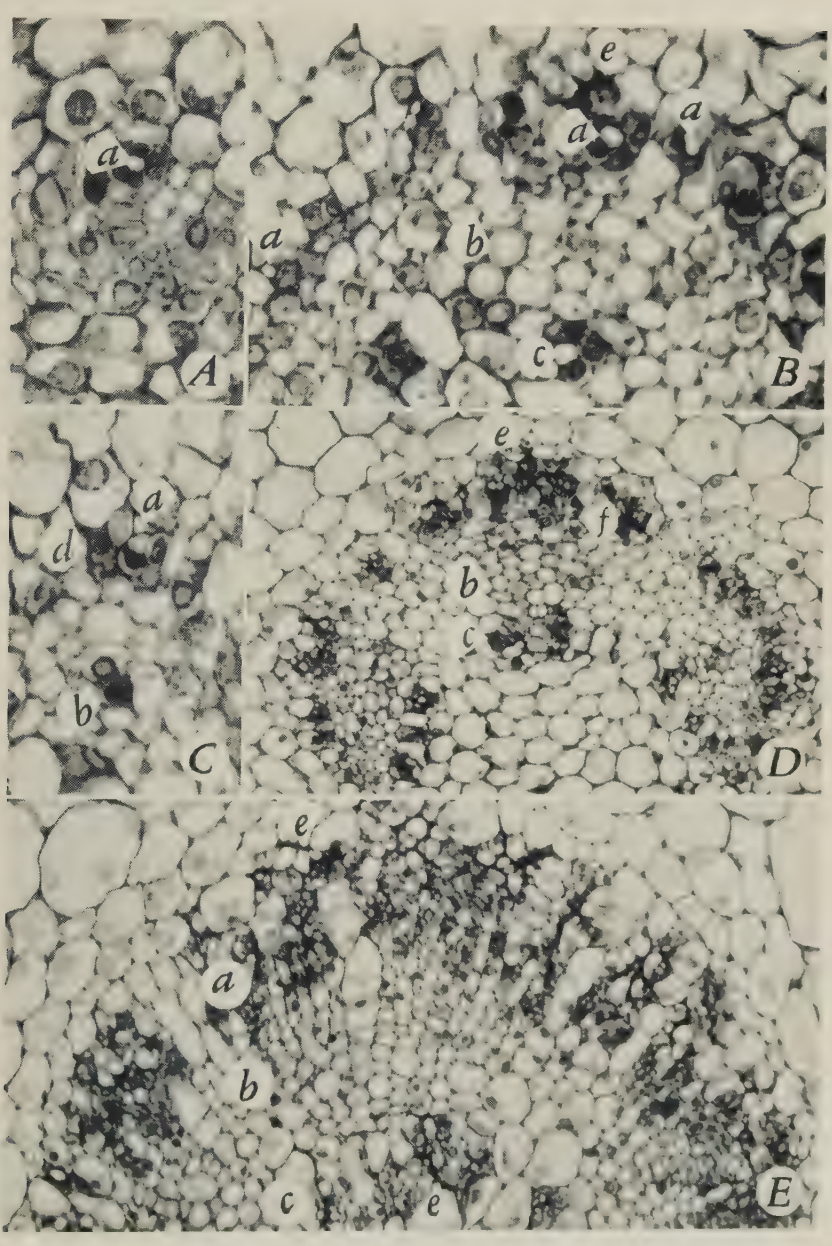

Fig. 14. Transverse sections of vascular tissues of leaves 2 (*A*), 3 (*C*), 4 (*B*), 5 (*D*), and 7 (*E*) from the same tomato shoot as shown in figure 13, *B*. Chromophily of cells surrounding the first sieve element (*a*) in *A*; early hyperplasia in *C* and *B*; hyperplasia and primary necrosis (*e*) in *D*; advanced hyperplasia in *E*. Details: *a*, sieve element and external phloem; *b*, xylem; *c*, internal phloem; *d*, locus of hyperplasia near sieve element; *e*, necrosis; *f*, hyperplasia in procambium. (*A–C*, × 780; *D*, *E*, × 255.)

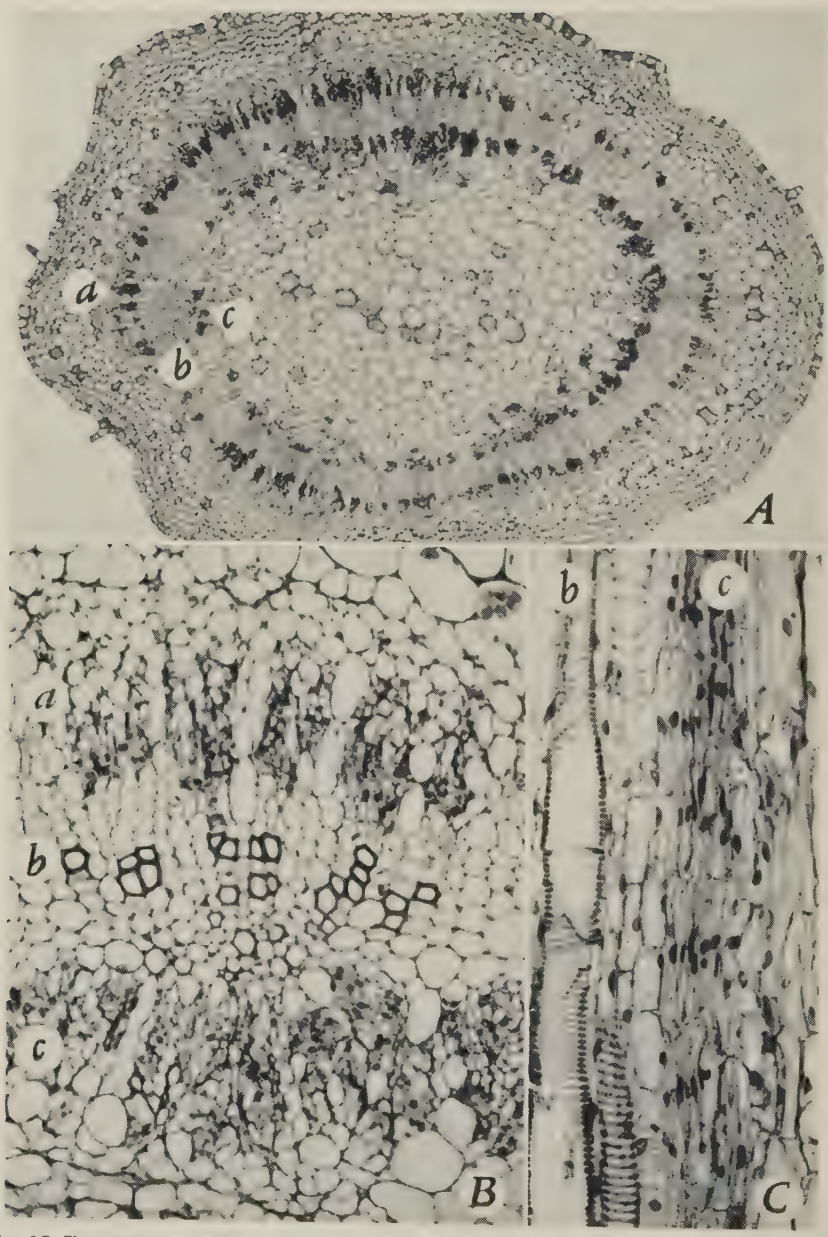

Fig. 15. Transverse (*A*, *B*) and longitudinal (*C*) sections of stem of aster yellows tomato plant in primary state of growth. Hyperplasia is present in external (*a*) and internal (*c*) phloem. Details: *a*, external phloem; *b*, xylem; *c*, internal phloem. (*A*, × 35; *B*, *C*, × 200.)

phloem strand to the left of the first mature sieve element contained an immature sieve element surrounded by chromophilic hyperplastic cells, so that the whole strand appeared rather homogeneous. No degeneration was evident to the right of the first mature sieve element. The internal phloem had an immature sieve element. The cells surrounding it were not hyperplastic, but chromophilic. One protoxylem element was mature and filled with gum (at *b* in fig. 14, *C*).

Leaf 4 (fig. 14, *B*) was the first leaf to show necrosis (at *e*). The sieve element in the median abaxial position of the external phloem had collapsed together with its neighboring cells in the centripetal direction. The cells right and left and some in the centripetal direction from the necrotic area were hyperplastic. The hyperplastic tissue had hypertrophied nuclei. The sieve elements that developed to the right and left of the first sieve element in the median abaxial position were all associated with hyperplasia. The internal phloem showed chromophily of the cells surrounding the first sieve elements (at *c*). The sieve elements did not become as clear as those in normal and curly top-diseased leaves. Six protoxylem elements were mature in the median position (fig. 14, *B*, at *b*).

In leaf 5 (fig. 14, *D*), necrosis (*e*) and hyperplasia were evident in the external and internal phloem. Necrosis was most conspicuous in the oldest parts of the phloem, but was involving the younger, hyperplastic tissue also. The hyperplastic cells were becoming obliterated a few cells at a time. Hyperplasia was spreading into the procambium (at *f*), but the cells derived from this procambium were arranged more regularly than in the curly top plants.

As in the curly top material, the hyperplastic tissue in aster yellows plants shows clearing of cells. However, there is no general clearing of masses of cells in aster yellows; instead, cleared cells appear singly or in small groups among densely-stained cells. Similarly, necrosis involves not large masses of the hyperplastic tissue, as in curly top, but small groups of cells (compare fig. 9, *A*, and fig. 14, *E*). There is a tendency in the aster yellows material for the necrosed areas to be aligned radially (fig. 14, *E*). Probably the two phenomena, the dispersed clearing of cells and the dispersed necrosis, are interrelated.

Figure 15 illustrates some features of phloem degeneration in stem sections of an aster yellows plant. The external (*a*) and internal (*c*) phloem regions show a similar intensity of hyperplasia in figure 15, *A*, rather than a greater intensity in the internal phloem as in figure 10, *A*, illustrating the effect of curly top. This difference is not regarded as constituting a difference between the two diseases in their effects upon the plant. Possibly, in the aster yellows plant, the timing in infection was different than in that infected with curly top.

Hyperplasia of primary ray tissue and depression of xylem differentiation, phenomena commonly observed in curly top, were absent or only weakly expressed in aster yellows plants.

The hyperplastic tissue in aster yellows plants differs in several features from that in curly top material. As already noted, this tissue is more irregular in cell arrangement in curly top plants than in aster yellows plants. In cross sections, the hyperplastic phloem in curly top forms more or less large nests that encroach upon the ground tissue among the phloem strands

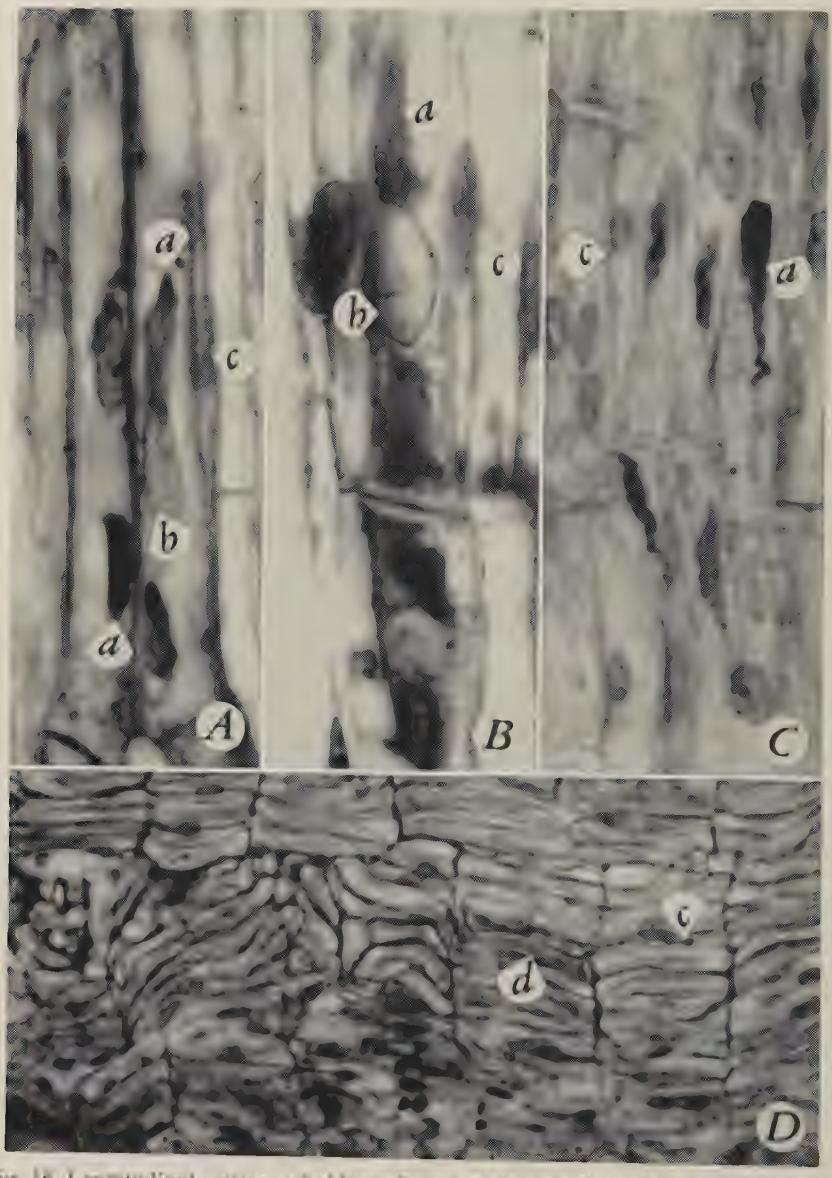

Fig. 16. Longitudinal sections of phloem from healthy (*A*, *B*) and aster yellows (*C*, *D*) tomato plants. *A* and *B*, young sieve elements with slime bodies (*a*). In *A*, the nuclei of the sieve elements are still chromatic, in *B*, highly vacuolate. *C* and *D*, hyperplastic phloem. Several slime bodies are discernible in *C*. The tissue in *D* shows some disturbance in cell arrangement, but clearly illustrates the characteristic radial zonation of cells. Details: *a*, slime body; *b*, nucleus; *c*, companion cell; *d*, sieve element. (*A*, *B*, × 1,080; *C*, ×780; *D*, × 490.)

(primary rays in the external phloem), and thus markedly changes the organization of the phloem tissue. In longitudinal sections, also, the cells vary greatly in shape and length and are haphazardly arranged (compare figs. 15, *C*, and 10, *C*; 16, *D*, and 9, *B*). In aster yellows, hyperplasia does not disturb the characteristic organization of the phloem into strands separated from one another by ground tissue (primary rays) (compare figs. 15, *B*, and 10, *B*), and the cells are more uniform in size and shape and are arranged in more or less definite radial series like products of a vascular cambium (fig. 16, *D*).

Another conspicuous difference is that most of the hyperplastic cells in curly top plants differentiate as abnormal sieve elements. In aster yellows, however, the tissue has a more normal appearance. The sieve elements often resemble normal sieve elements in shape and size, have companion cells, and are accompanied by parenchyma cells (compare figs. 16, *D*, and 9, *B*). The main morphologic difference between the phloem in normal and aster yellows shoots is that the tissue is more massive in the diseased material and that it undergoes necrosis. The sieve elements of the hyperplastic phloem have slime bodies in their early stages of development (fig. 16, *C*). The nuclei and slime bodies of the sieve elements of the hyperplastic phloem stain pink with Giemsa stain.

The occurrence of parenchyma and companion cells (fig. 16, *D*) in the hyperplastic phloem in aster yellows infection explains why the clearing of the tissue is dispersed in contrast to the general clearing of the abnormal phloem in curly top plants. In relation to this difference, necrosis in curly top involves large masses of cells, and the surrounding cells hypertrophy and divide (wound-healing reaction), closing the potential lacunae (fig. 9, *A*). The dispersed condition of necrosis in aster yellows, resulting from the presence of parenchyma cells that do not collapse, is not accompanied by an intense wound-healing reaction (figs. 14, *E*, and 15, *B*).

Phloem Degeneration in the Root. As in curly top-affected plants, the basic anatomic structure of roots of plants infected with aster yellows is not substantially different from normal. The roots, like the shoots, show depression of growth at the apical meristem although, in both, the degenerative changes are localized in the phloem. Some roots show degeneration next to the apical meristem (fig. 5, *C*) because, in connection with the pronounced cessation of growth, the first sieve elements mature close to the apex. This type of root is not suitable for study of the stages of phloem degeneration. Instead, less severely affected roots, with the first sieve element found about 260 microns from the apex and with some meristematic activity at the apex, should be used. As with curly top, the stages of phloem degeneration induced by aster yellows were traced from the tip to the more mature region of the root. Two semiactive roots (fig. 17, *A*, *C*, *E*) were selected for this purpose. In addition, sections from four other roots were used to show details of degeneration at higher magnification in figures 17, *B*, *D*, *F*, and 18, *A* to *D*.

Root tips from aster yellows plants, like those from some curly top plants, show a higher degree of vacuolation of meristematic cells than do normal root tips, and therefore stain rather lightly. In general, this evidence of depressed growth is more common and more pronounced in aster yellows than in curly top roots. If the depression of growth is pronounced,

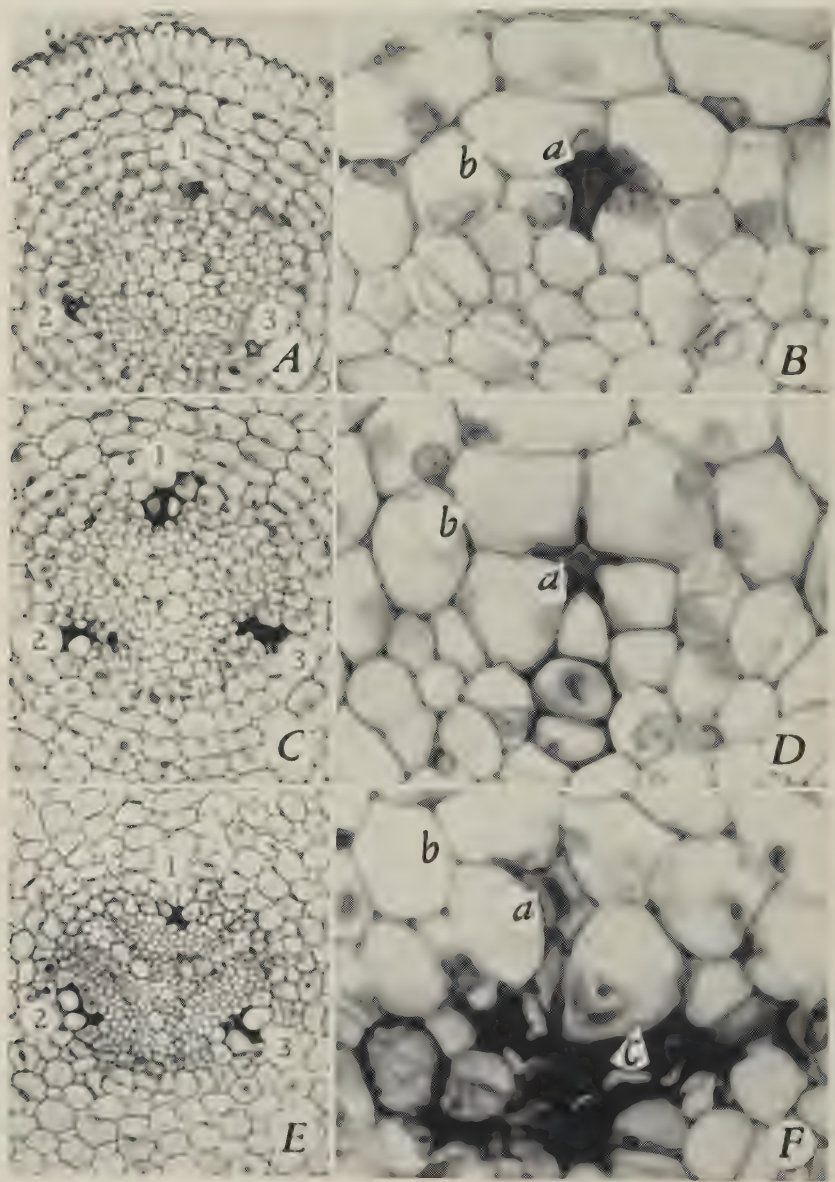

Fig. 17. Transverse sections of roots of aster yellows tomato plants. The sections in *A*, *C*, and *E* were taken 300, 470, and about 1,200 microns from the apex. The numbers 1–3 indicate the phloem poles. Progressive degeneration of sieve elements and adjacent cells is seen at each pole in *A*, *C*, and *E*. *B*, *D*, *F*, single phloem poles. The protophloem sieve element (*a*) is occluded by gum in *B*, is partly crushed in *D* and *F*. *F* also shows secondary necrosis and, at *c*, secondary hypertrophy. Details: *a*, protophloem sieve element; *b*, pericyclic cell; *c*, hypertrophied cell. (*A*, *C*, *E*, × 230; *B*, *D*, *F*, × 1,000.)

the vascular elements mature close to the apical meristem and the Casparian strips develop as soon as the endodermis is formed near the apex.

In figure 17, *A*, at a level 270 microns from the apex, the three sieve elements, one at each pole, are seen to contain gum. One such occluded sieve element appears at higher magnification in figure 17, *B*. The nucleus was still present in this element but was obscured by the very densely-stained, degenerated cytoplasm.

About 300 microns from the apex (fig. 17, *C*), the degeneration had spread from the sieve elements to the neighboring cells, especially those in the pericycle. The sieve elements remained occluded—in fact, they did not become clear even at lower levels. In more severe cases degeneration affected the cortex also (fig. 5, *C*). The cells adjacent to the sieve tube usually appeared to die (primary necrosis) without undergoing the gradual cytoplasmic changes and without developing the variously shaped bodies characteristic of the degenerating cells in curly top (compare figs. 17, *A*, *C*, *E*, and 11, *A*, *B*). Roots obtained by rooting of stems affected with aster yellows showed less rapid death of cells, and the gradual degenerative changes in the cytoplasm were similar to those in curly top-diseased roots. The sieve tubes appeared to mature normally, and the surrounding cells showed cytoplasmic bodies and fibrous cytoplasm (fig. 18, *B*). It should be noted that these roots showed no other abnormalities than these cytoplasmic changes in the cells around the sieve elements. The adventitious roots, which grew spontaneously on the stems of plants affected with aster yellows, were usually as severely degenerated as the branch roots of the taproot system (e.g., fig. 18, *E*).

As stated earlier, in severely affected roots of aster yellows plants the sieve elements commonly do not become clear, and they eventually collapse in gum-occluded condition (figs. 17, *D*, and 18, *D*). Later, some hyperplasia occurs in the centripetal direction, that is, in the procambium normally developing into metaphloem (figs. 18, *D*, and 19, *B*). In contrast to the hyperplastic tissue in curly top roots, in which the cells are haphazardly arranged (fig. 19, *C*), that in aster yellows roots shows regular divisions, and the cells occur in blocks of radial series (fig. 19, *B*). Some of these cells acquire the properties of sieve elements by developing sieve areas. They also become enucleate and at least partially clear—features indicating that these cells are sieve elements similar to those found in the hyperplastic tissue in the shoot. No slime bodies were encountered in the hyperplastic tissue, probably because too few sieve elements were in immature stages in the sections. Some nucleate cells associated with the abnormal sieve elements appeared to be companion cells, others parenchyma cells. They were of the same size and shape as the sieve elements. As a whole, the hyperplastic tissue stained more densely than the normal phloem (compare *A* and *B* in fig. 19).

The hyperplastic tissue usually collapses one or a few cells at a time, and sometimes in a large mass, as in roots of a curly top plant, but lacunae are not formed (fig. 17, *E*, *F*). When the collapse involves many cells at once, some cells in the obliterated area enlarge and occupy the space left by the collapsed cells (fig. 17, *F*, at *c*). Such wound-healing reaction (see-

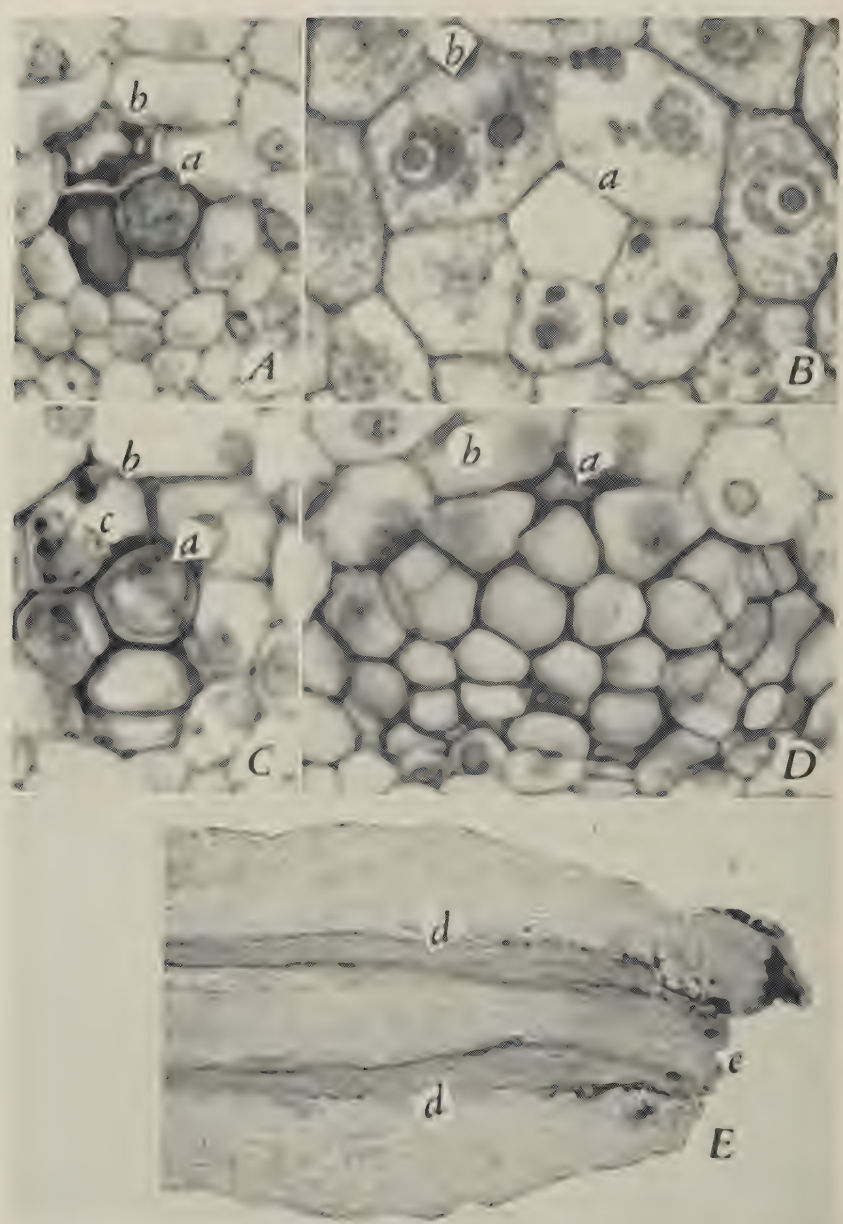

Fig. 18. Roots of aster yellows tomato plants. *A–D*, single phloem poles in transverse sections. The first sieve element (*a*) is immature in *A* and *C*, mature in *B*; cells next to this element show various degenerative changes. In *D*, the protoxylem is partly collapsed and the metaphloem below it is slightly hyperplastic. *E*, longitudinal section of severely affected root tip with the apical meristem completely degenerated (*e*). Details: *a*, protophloem sieve element; *b*, pericycle cell; *c*, crystal; *d*, hyperplastic phloem; *e*, root apex. (*A–D*, × 1,600; *E*, × 32.)

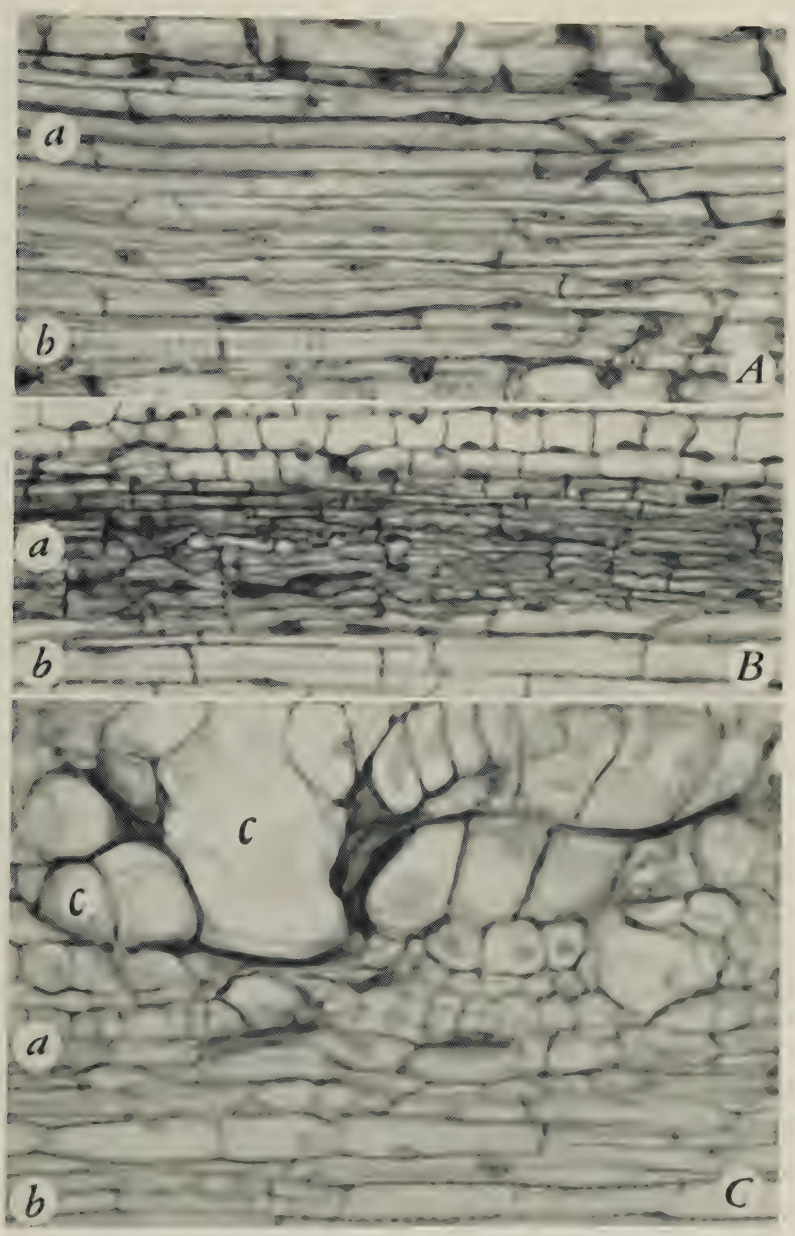

Fig. 19. Longitudinal sections of tomato roots in primary state of growth from healthy (*A*), curly top (*B*), and aster yellows (*C*) plants. Hyperplastic phloem in *B*, edge of phloem and wound-healing hypertrophy and hyperplasia in *C*. Details: *a*, phloem; *b*, xylem; *c*, cortex undergoing hypertrophy and hyperplasia. (All × 390.)

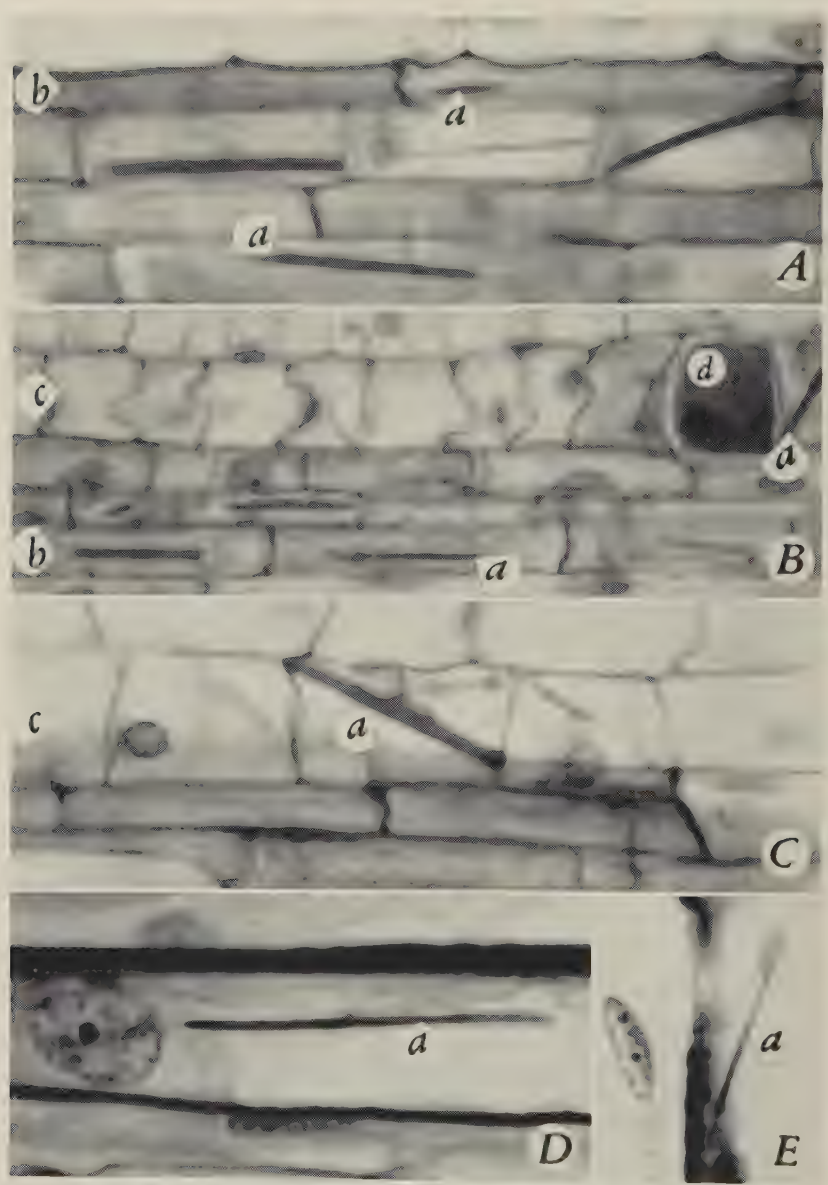

Fig. 20. Longitudinal sections of roots of tomato (*A*, *B*, *C*) and stem of *Nicotiana rustica* (*D*, *E*) showing crystals found in aster yellows infections. Details: *a*, crystal; *b*, series of [illegible] sieve elements; *c*, [illegible] cells; *d*, [illegible] cell with crystal. (*A*, *C*, × 1,400; *B*, × 880; *D*, *E*, × 780.)

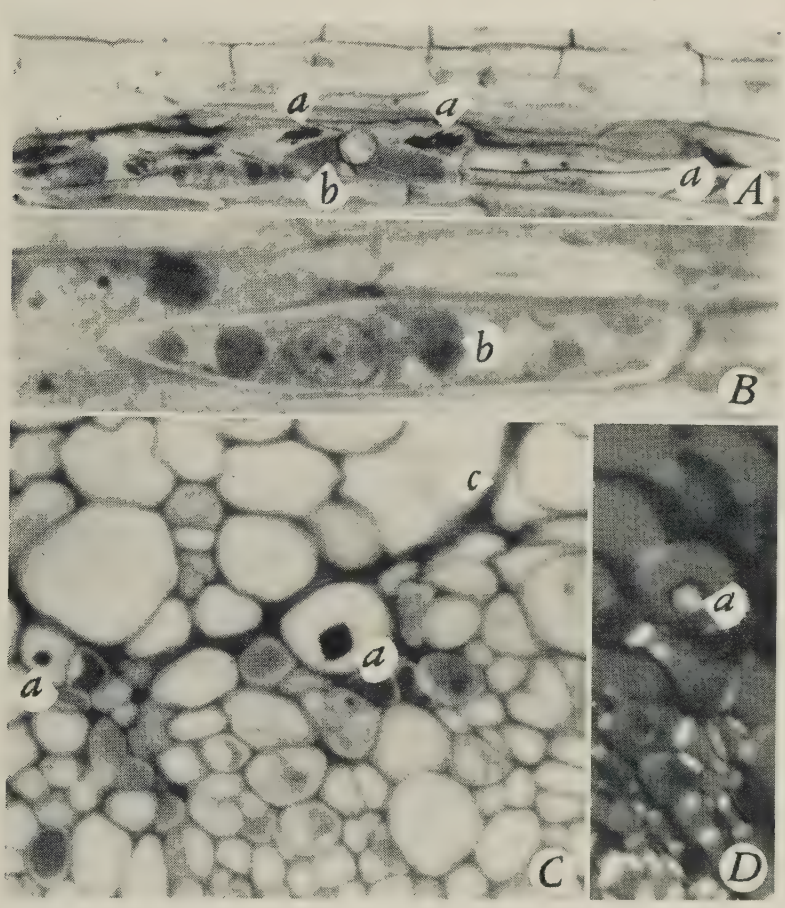

Fig. 21. Longitudinal (*A*, *B*) and transverse (*C*, *D*) sections of vascular bundles of *Vinca* infected with aster yellows, showing inclusions in phloem-parenchyma cells. *D*, photographed with polarized light; double refraction shown by crystal (*a*) and secondary walls in xylem (below). Details: *a*, crystals; *b*, inclusion, cytoplasmic in nature; *c*, necrosed phloem. (*A*, × 490; *B–D*, × 1,400.)

ondary hypertrophy) is usually more pronounced in curly top roots (fig. 19, *C*, at *c*).

Inclusion Bodies. In some collections of roots from the tomato plants infected with aster yellows, crystalline inclusions were found in various cells of the degenerated phloem. Since such inclusions were not previously described in connection with the aster yellows disease, a survey of occurrence of these inclusions was extended to several other hosts of the virus. The following species, experimentally infected with several Western strains of aster yellows virus by means of the leafhopper vector, were kindly supplied by Dr. J. H. Freitag: *Apium graveolens* L. (celery), *Callistephus chinensis* Nees (aster), *Linum usitatissimum* L. (flax), *Nicotiana rustica* L. (tobacco), *Plantago major* L. (plantain), and *Vinca rosea* L. (periwinkle). Young root and shoot parts were examined in fresh material and in prepared slides.

Inclusions were found in all the species listed. The different samples varied greatly in numbers of crystals present, and some had none at all although the degenerative changes in the phloem were conspicuous. Remarkably enough, inclusions were found in both root and shoot parts in all species, except in tomato; in this species the crystals were detected only in the roots.

The inclusions typically appear as elongated, slender bars pointed at one end and truncate at the other (fig. 20). They vary in length, and in shorter cells may equal or exceed the long diameter of the cell, so that they assume an inclined position (fig. 20, *A*, *C*). In transverse views the crystals are diamond-shaped (fig. 21, *C*). Two observations indicate that the diamond-shaped structures are indeed transverse sections of long crystals. When such a structure is found in a cell in a cross section of phloem it may be followed through several sections of the same cell; in other words, it is an elongated structure. When the crystal appears in a longitudinal but slightly inclined position, viewing the truncate end at different focal planes reveals the diamond shape.

The crystals were not completely investigated with regard to their chemistry. They were found to be soluble in HCl and H_2SO_4. Saturated picric acid, acetic acid, benzene, and chromic acid had no visible effect. They stained bright red with safranin, but remained colorless when treated with trypan blue or trypan blue and chlorazol pink (McWhorter, 1957). They were not tested with the eosin or Giemsa stains, the Feulgen technique, or Millon's reagent.

In occasional sections, fresh and processed, it was possible to detect a weak double refraction in longitudinal and transverse views. The double refraction of the crystal (fig. 21, *D*, at *a*) was weaker than that of the secondary wall of the xylem elements (fig. 21, *D*, below).

Although the crystalline inclusions are typically long bars, many cells contain shorter inclusions of the same shape as the long ones, and still shorter ones whose shape could not be properly identified. The long crystals are usually single in a cell, or occur in pairs, the short ones appear in various quantities—often numerous and disposed in clusters (fig. 21, *A*). Sometimes short bodies are aligned in such a way as to suggest a broken crystal. (The long crystals were found to break easily in fresh sections when pressure was exerted on the cover glass.) Sometimes a long crystal appears considerably thinned down (fig. 20, *E*), with the margins frayed as though corroded.

All these bodies stain bright red with safranin. Tentatively, they all are assumed to be the same material appearing in different forms.

The exact location of the crystals in the protoplast has not been determined. Sometimes they appear to be in contact with the nucleus (fig. 20, *C*), sometimes confined to the vacuole (figs. 20, *D*; 21, *A*, small crystals).

With regard to the types of cells containing the crystals, most of them were various parenchyma cells in the phloem itself and in the ground tissue next to the phloem. Some of these cells showed no visible degeneration of the protoplast (fig. 20, *D*), others had the dense contents so common in degenerating cells in virus-diseased plants (figs. 20, *B*, at *d*; 21, *A*, at *b*). They were present in cells that were still intact and in those that were crushed and more or less completely collapsed (as those at *c* in fig. 21, *C*).

Special effort was made to study the relation of the crystals to the sieve elements. In young root parts of tomato they may be found in parenchyma cells (pericyclic and others) next to the first sieve elements (fig. 18, *C*). They were detected in companion cells in shoot parts of periwinkle. They were also found in sieve elements of periwinkle and tomato—elements that still had nuclei and those that were devoid of nuclei (row of cells marked *b* in fig. 20, *A*, *B*).

In addition to the crystalline inclusions, many cells had various cytoplasmic masses ranging from formless clumps of deeply staining material (fig. 21, *A*, at *b*) to globular bodies (figs. 18, *B*; 21, *B*, at *b*). These bodies were mentioned previously and were compared with similar bodies in curly top-diseased material. They are referred to again to point out their difference from the crystalline inclusions. They appear to be cytoplasmic in texture and staining reactions, except when the cell is necrosed. Then the masses become more or less homogeneous, and stain red or orange-red; that is, they assume the appearance of wound gum.

DISCUSSION

The present anatomical study on tomato agrees with the previous findings, on curly top and aster yellows (Artschwager and Starrett, 1936; Esau, 1935*a*, *b*, 1941, 1957; Girolami, 1955), that on anatomic grounds, both viruses may be interpreted as phloem-limited viruses and that they induce hypertrophy, hyperplasia, and necrosis in the phloem tissue. The close relation of the curly top virus to the phloem has also been demonstrated by numerous critical physiological experiments (cf. Esau, 1956). Such information is not available for the aster yellows virus.

The present study also confirms Girolami's (1955) conclusion that the effects of the curly top and the aster yellows viruses on the phloem tissue of the host are very similar; the differences are mainly those of detail of development of the degenerative changes and of the final organization of the affected phloem. These details, however, permit differentiation of the two viruses; and in the present study, the Western strain of aster yellows was differentiated from curly top by the discovery of crystalline inclusions in aster yellows plants.

The apparent dependence of the aster yellows and curly top viruses on differentiated sieve elements for their vertical spread—phloem symptoms occur only in organs that have mature sieve elements (e.g., Esau, 1941;

Girolami, 1955)—was observed in the tomato also. The characteristic spread of symptoms in relation to maturation of the sieve elements in young plant parts does not necessarily indicate that the viruses are localized in the sieve elements and their vicinity. Lackey (1946), for example, reported a high concentration of the curly top virus in the apices of roots of beet and tobacco, although no symptoms were observed in the apical meristems.

With regard to the spread of symptoms along mature sieve tubes, the aster yellows disease shows a slight deviation from the curly top disease. In tomato affected by aster yellows, chromophilic cells, that is, cells in incipient stages of degeneration, may appear next to sieve elements that are still nucleate and hence immature. The crystalline inclusions found in aster yellows may also be observed in immature phloem. Curly top appears to be more dependent on mature sieve elements for the spread of its symptoms than is aster yellows, for degeneration of cells has been seen only in association with mature sieve elements.

Both curly top and aster yellows viruses may induce degeneration in long series of immature sieve elements which are continuous with mature elements. It thus appears that the virus is translocated from differentiated sieve elements to immature sieve elements and induces degeneration in the latter. Another possibility might be that some kind of obstruction occurs in the differentiated elements that prevents movement into the immature sieve elements of some materials necessary for sieve-element differentiation. Such failure of the young elements to mature normally may lead to their degeneration.

Although the distinction between primary and secondary degenerative changes in the phloem, as proposed by Esau (1935*a*) for curly top, is applicable to the aster yellows infection (Girolami, 1955; and the present study), in the latter the separation into the various stages is less sharp than in curly top, and primary hypertrophy (enlargement of cells in immediate proximity to the first mature sieve elements) has not been discerned in tomato. These are minor deviations, however. The general sequence of degeneration is the same in both diseases. The first major change is a degeneration of cells—chromophily with or without primary hypertrophy—next to the normally-matured sieve elements. These cells may become necrosed (primary necrosis). Other cells in the neighborhood of the sieve elements undergo primary hyperplasia. When the hyperplastic cells become necrosed, the stage of secondary necrosis is reached. The wound-healing reactions occurring in response to the primary and secondary necrosis involve secondary hypertrophy and hyperplasia. In view of the possible absence of primary hypertrophy, but a consistent presence of initial chromophily, the sequence for both diseases may be revised as follows: primary chromophily with or without primary hypertrophy; primary necrosis; primary hyperplasia; secondary necrosis; secondary hypertrophy and hyperplasia. In both diseases, of course, the stages overlap in the same region, and they can be distinguished only by developmental studies.

The hyperplastic phloem in aster yellows tomato deviates less from the phloem of the normal plant than does that in curly top tomato. The sieve elements are considered abnormal, however, because they occur in larger numbers than in healthy plants, and undergo necrosis. They are rather

regularly arranged and are associated with companion cells and parenchyma cells. They appear to clear a few cells at a time and also become obliterated gradually. Most of the parenchyma cells do not become obliterated, so that the necrotic regions are relatively small and scattered. In curly top infection, the hyperplastic tissue is composed mainly of abnormal sieve elements which are irregularly arranged; and companion cells and parenchyma cells appear to be absent. Obliteration involves large masses of cells rather than a few cells at a time, such as takes place with aster yellows. These histologic differences indicate that curly top is more virulent than aster yellows with regard to the tomato plant.

The present study confirms Girolami's (1955) findings that both viruses induce the differentiation of numerous abnormal sieve elements. Special attention was given to the early stages of development of these elements in order to clarify the identity of the bodies resembling normal slime bodies so prevalent in the hyperplastic tissue. The bodies in question were found to resemble the normal slime bodies in every developmental detail, including their dispersal at the time of nuclear breakdown.

When treated according to Giemsa (cf. Rawlins and Takahashi, 1952), the slime bodies stain pink in normal tomato plants and in those infected with curly top and aster yellows. A similar staining reaction was observed in the slime of mature sieve elements in several species of plants, notably clearly in *Cucurbita*. Thus, the conclusion that the bodies in the hyperplastic tissue are slime bodies and that the cells containing them are more or less abnormal sieve elements is fully justified.

In contrast to curly top, aster yellows causes pronounced retardation and even a stoppage of growth in the apical meristem. As a result, the vascular tissues mature closer to the apex in both the shoot and the root of aster yellows plants than they do in plants affected with curly top and in normal plants. In severe cases of cessation of growth, the sieve elements mature almost at the very apex. Therefore, if degeneration is found in the apical region, it occurs there because of the proximity of mature sieve elements.

In roots of aster yellows and curly top plants that have stopped growing, the Casparian strip is found as soon as the endodermis is formed. A similar situation was observed by Wilcox (1954) in the dormant roots of *Abies*. In addition to the normal endodermis, Wilcox found, in the dormant roots, a secondary endodermis with suberin lamellae that enclosed the entire apex. It may be concluded, therefore, that the stoppage of meristematic activity in the roots brings about a differentiation of tissues close to the apex regardless of whether this stoppage results from effects of viruses, or environmental conditions, or some internal factors of growth.

Crystal-like inclusions, not previously described, were found in many roots of tomato infected with a Western strain of aster yellows. No such inclusions were observed in the shoot parts. In several other species infected with strains of aster yellows virus the crystalline inclusions occurred in both shoot and root. Among these species was flax (*Linum usitatissimum*), also used by Girolami (1955). The latter found no crystals in aster yellows shoots of flax. These variations in the occurrence of crystals defy explanation for the present. Perhaps the development of the crystalline inclusions depends on some specific condition of the cell, a condition that may be absent at times.

The literature was not searched exhaustively with regard to information on inclusion bodies resembling those found in the aster yellows plants. Garjeanne (1918) and Brat *et al.* (1951) described rod-like crystals, which they called rhabdoids, in supposedly healthy *Drosera*. Such rhabdoids are generally assumed to be proteinaceous. The rhabdoids of *Drosera* are described as labile, easily becoming deformed when injured. They are said to round off into small droplets under certain conditions. The crystals in aster yellows plants are not labile. When subjected to pressure they break rather than round off, and they may be flushed out and made to float in a mounting solution with retention of form. Like calcium-oxalate crystals, they dissolve in HCl and H_2SO_4, but unlike calcium oxalate they stain red with safranin.

The crystals are obviously helpful in distinguishing the aster yellows from the curly top infection, and may indicate different kinds of disturbance of metabolism in the two diseases. On the other hand, the crystals might have a close relation to the virus itself. They merit further study.

As mentioned in the introduction, the external symptoms of curly top and aster yellows are usually difficult to distinguish. The greenhouse plants used in the present study showed some differences. Aster yellows tomato produced numerous adventitious roots, a reaction not observed in curly top tomato (cf. also Esau, 1941). According to Girolami (1955), in flax both viruses produced quite similar symptoms—yellowing and curling of young leaves. Girolami recorded no stimulation of adventive root formation. It should be remembered, however, that in contrast to flax, tomato normally has adventitious root primordia in the mature stems. These primordia may be induced to grow by application of certain growth hormones (Zimmerman and Wilcoxon, 1935). The observations on aster yellows tomato suggest that the virus, like certain growth hormones, is capable of stimulating dormant adventitious root primordia to grow.

Tomato plants infected with aster yellows by grafting also produced numerous lateral shoots. According to Smith (1957, p. 40), the stimulation of development of lateral shoots differentiates the aster yellows disease from curly top in tomato. This statement, however, is based on studies with Eastern strains of aster yellows. Thus far, production of lateral shoots in tomato infected with Western strains of aster yellows has not been recorded (personal communication by Dr. J. H. Freitag). Possibly the use of relatively old plants, removal of terminal bud, and inoculation by grafting (rather than by leafhoppers) had some effect on symptom development in plants used in the present study.

SUMMARY

The curly top virus was found to induce degenerative changes in the phloem of tomato similar to those previously reported for several other plants. In both the root and the shoot parts, the degenerative changes were observed only at the level where a sieve element had matured. The first degenerative changes usually consisted of primary chromophily, and sometimes of primary hypertrophy, of cells adjacent to the mature sieve element. Series of immature sieve elements themselves sometimes became chromophilic; then the adjacent cells remained seemingly normal. The

degenerated cells adjacent to sieve elements became necrosed (primary necrosis). Before and after this necrosis, hyperplastic divisions occurred in cells nearby (primary hyperplasia). Such divisions resulted in the production of numerous short, haphazardly arranged cells which were interpreted as abnormal sieve elements. Parenchyma and companion cells were not identified among these cells. The abnormal sieve elements differed from the normal elements mainly in size, shape, and arrangement; otherwise they resembled sieve elements. They passed through developmental stages similar to those of the normal sieve elements, and became enucleate. Eventually the hyperplastic tissue became necrosed and collapsed as a mass (secondary necrosis). In the shoot, the neighboring cells of the collapsed tissue divided (secondary hyperplasia) and occupied the space left by the collapsed cells; hence no lacunae were produced. In the root, however, such division was sometimes sluggish, and lacunae were formed. Xylem differentiation was much suppressed since cells which normally differentiate into tracheary elements also became hyperplastic and formed abnormal sieve elements. Thus, abnormal sieve elements were found among xylem elements. Mature phloem tissue was not noticeably affected by the disease.

Aster yellows virus induced degenerative changes in the phloem of tomato similar to those previously reported for flax. Moreover, these degenerative changes were in many respects similar to those resulting from curly top infection. One of the deviating features was that degenerative changes were found at the level where the sieve elements were still immature. These changes consisted of primary chromophily and primary hyperplasia of the cells adjacent to the immature sieve element. Series of immature sieve elements sometimes became chromophilic. The cells adjacent to these affected sieve elements degenerated also. The hyperplastic divisions resulted in the production of numerous cells which were similar to normal phloem cells in size, shape, and arrangement, but differed in numbers. Most of these cells were abnormal sieve elements, but they passed through developmental stages similar to those of the normal sieve elements. Moreover they were associated with parenchyma and companion cells. Xylem differentiation was not affected; in fact the procambium appeared to be somewhat stimulated, and numerous narrow tracheary elements were found in severely affected leaves. In aster yellows plants the degenerative stages, the primary and secondary necrosis and hyperplasia, were not so clearly distinguishable as in curly top infections. Moreover, the abnormal sieve elements differentiated in a more orderly sequence, a few cells at a time, and were obliterated more gradually than in curly top plants. No lacunae were formed. Mature phloem tissue was not noticeably affected by the virus. Crystal-like inclusions were found associated with the phloem in the roots of tomato infected with a Western strain of aster yellows. Similar inclusions were found in the roots and shoots of *Apium graveolens* L., *Linum usitatissimum* L., *Nicotiana rustica* L., *Plantago major* L., and *Vinca rosea* L. infected with Western strains of aster yellows.

LITERATURE CITED

ARTSCHWAGER, E. F.

1918. Anatomy of the potato plant, with special reference to the ontogeny of the vascular system. Jour. Agr. Res. **14**: 221–52.

ARTSCHWAGER, E. F. and R. C. STARRETT

1936. Histological and cytological changes in sugar-beet seedlings affected with curly top. Jour. Agr. Res. **53**: 637–57.

BEDESEM, P. P., Jr.

1958. Histogenetic effects of 2,3,5 triiodobenzoic acid [TIBA] on the shoot apices and leaf primordia of tomato. Torrey Bot. Club Bul. **85**: 434–72.

BENNETT, C. W.

1934. Plant-tissue relations of the sugar-beet curly-top virus. Jour. Agr. Res. **48**: 665–701.

1937. Correlation between movement of the curly top virus and translocation of food in tobacco and sugar beet. Jour. Agr. Res. **54**: 479–502.

1943. Influence of contact period on the passage of viruses from cion to stock in Turkish tobacco. Phytopathology **33**: 818–22.

1944. Studies of dodder transmission of plant viruses. Phytopathology **34**: 905–32.

BENNETT, C. W., and K. ESAU

1936. Further studies on the relation of the curly top virus to plant tissues. Jour. Agr. Res. **53**: 595–620.

BRAT, L., G. KENDA, and F. WEBER

1951. Rhabdoide fehlen den Schliesszellen von *Drosera*. Protoplasma **40**: 633–35.

CHEADLE, V. I., E. M. GIFFORD, Jr., and K. ESAU

1953. A staining combination for phloem and contiguous tissues. Stain Technol. **28**: 49–53.

CRAFTS, A. S.

1933. Sieve-tube structure and translocation in the potato. Plant Physiol. **8**: 81–104.

1934. Phloem anatomy in two species of *Nicotiana*, with notes on the interspecific graft union. Bot. Gaz. **95**: 592–608.

CURRIER, H. B., and S. STRUGGER

1956. Aniline blue and fluorescence microscopy of callose in bulb scales of *Allium cepa* L. Protoplasma **45**: 552–59.

ESAU, K.

1933. Pathologic changes in the anatomy of leaves of the sugar beet, *Beta vulgaris* L., affected by curly top. Phytopathology **23**: 679–712.

1935*a*. Ontogeny of the phloem in sugar beets affected by the curly-top disease. Amer. Jour. Bot. **22**: 149–63.

1935*b*. Initial localization and subsequent spread of curly-top symptoms in the sugar beet. Hilgardia **9**(8): 395–436.

1938. Ontogeny and structure of the phloem of tobacco. Hilgardia **11**(8): 343–424.

1941. Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia **13**(8): 437–90.

1944. Anatomical and cytological studies on beet mosaic. Jour. Agr. Res. **69**: 95–117.

1956. An anatomist's view of virus diseases. Amer. Jour. Bot. **43**: 739–48.

1957. Curly top symptoms in an inoculated cotyledon of the sugar beet. Hilgardia **27**(1): 1–14.

GARJEANNE, A. J. M.

1918. Die Rhabdoide von *Drosera rotundifolia* L. Rec. Trav. Bot. Néerland. **15**: 237–54.

GIROLAMI, G.

1955. Comparative anatomical effects of the curly-top and aster-yellows viruses on the flax plant. Bot. Gaz. **116**: 305–22.

HAYWARD, H. E.

1938. The structure of economic plants. New York: The Macmillan Company. 674 pp.

LACKEY, C. F.

1946. Occurrence of curly-top virus in meristematic tissue. Phytopathology **36**: 462–68.

1952. Anatomical studies of injury of sugar beets induced by three strains of curly-top virus. Proc. Amer. Sugar Beet Technol. **1952**: 533–35.

McWhorter, F. P.
1957. Chlorazol fast pink B and trypan blue tests for [plant] virus and other proteinaceous inclusions. Stain Technol. **32**: 135–38.
Rawlins, T. E., and W. N. Takahashi
1952. Technics of plant histochemistry and virology. Millbrae, California: National Press. 67 pp.
Sass, J. E.
1958. Botanical microtechnique. 3d ed. Ames, Iowa: Iowa State College Press. 228 pp.
Severin, H. H. P.
1929. Curly top symptoms on the sugar beet. California Agr. Exp. Sta. Bul. **465**.
Smith, K.
1957. Textbook of plant virus diseases. 2nd ed. London: J. and A. Churchill. 652 pp.
Went, F. W.
1944. Morphological observations on tomato plant. Torrey Bot. Club Bul. **71**: 77–92.
Wilcox, H.
1954. Primary organization of active and dormant roots of noble fir, *Abies procera*. Amer. Jour. Bot. **41**: 812–21.
Zimmerman, P. W., and F. Wilcoxon
1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Boyce Thompson Inst. Contrib. **7**: 209–29.

STUDIES ON PENETRATION OF SUGAR BEET LEAVES BY STYLETS OF *MYZUS PERSICAE*[1]

K. ESAU,[2] R. NAMBA,[3] and E. A. RASA[4]

INTRODUCTION

THE PRESENT STUDY was undertaken as a corollary to studies on various aspects of beet yellows, a viral disease the causative agent of which is transmitted by *Myzus persicae* (Sulzer) and certain other aphids. As judged by the transmission and translocation of the beet yellows virus (Bennett, 1960)[5] and the histologic changes induced in the host (Esau, 1960), the virus is transported through the plant in the phloem but is not strictly limited to that tissue.

According to Roberts (1940), Sylvester (1956), and Bennett (1960), *M. persicae* is more successful in transmitting the virus during long periods of penetration of leaves than during short ones, and Roberts and Bennett suggest that this difference may depend on whether or not the insect reaches the phloem. Roberts (1940) examined 39 stylet penetration tracks of *M. persicae*, 18 produced in 15-minute periods, 12 in 2-hour periods, and 9 in 24-hour periods, and found that only 2 out of 18 reached the phloem in the 15-minute set, 6 out of 12 and 7 out of 9 in the longer-lasting penetrations. Some preliminary studies on the feeding habit of *M. persicae* led Bennett (1960) to conclude that this aphid feeds predominantly in the phloem. To test this concept further, the present study was undertaken.

MATERIAL AND METHODS

Noninfective *Myzus persicae* and healthy sugar beet (*Beta vulgaris* L.) seedlings with the first two foliage leaves partly or fully expanded were used. The insects were made to fast for at least an hour before they were placed on one of the first two leaves. The leaf material on which the insects had fed was prepared for microscopic study by a paraffin-embedding method involving a fixation in formalin-acetic-alcohol (Sass, 1958, p. 15), dehydration through tertiary butyl alcohol, and embedding in paraffin. The sections were cut 10 microns thick, and stained with hematoxylin and safranin. Since the aim was to survey a large number of pentrations, the simplest course was taken: the penetrations were studied by reference to the saliva sheaths left in the tissues by the insect stylets. With the staining combination employed, the sheaths were stained bright red.

The penetrations were obtained by two methods. In one, groups of insects were placed on a single leaf and killed after the desired time interval had passed. The leaf material was then fixed immediately. This material was prepared and collected by Dr. C. W. Bennett in a greenhouse at Salinas, Cali-

[1] Submitted for publication September 19, 1960.

[2] Professor of Botany and Botanist in the Experiment Station, Davis.

[3] Associate Entomologist, Agricultural Experiment Station, University of Hawaii, Honolulu.

[4] Formerly Research Assistant, Department of Botany, Davis.

[5] See "Literature Cited" for citations referred to in the text by author and date.

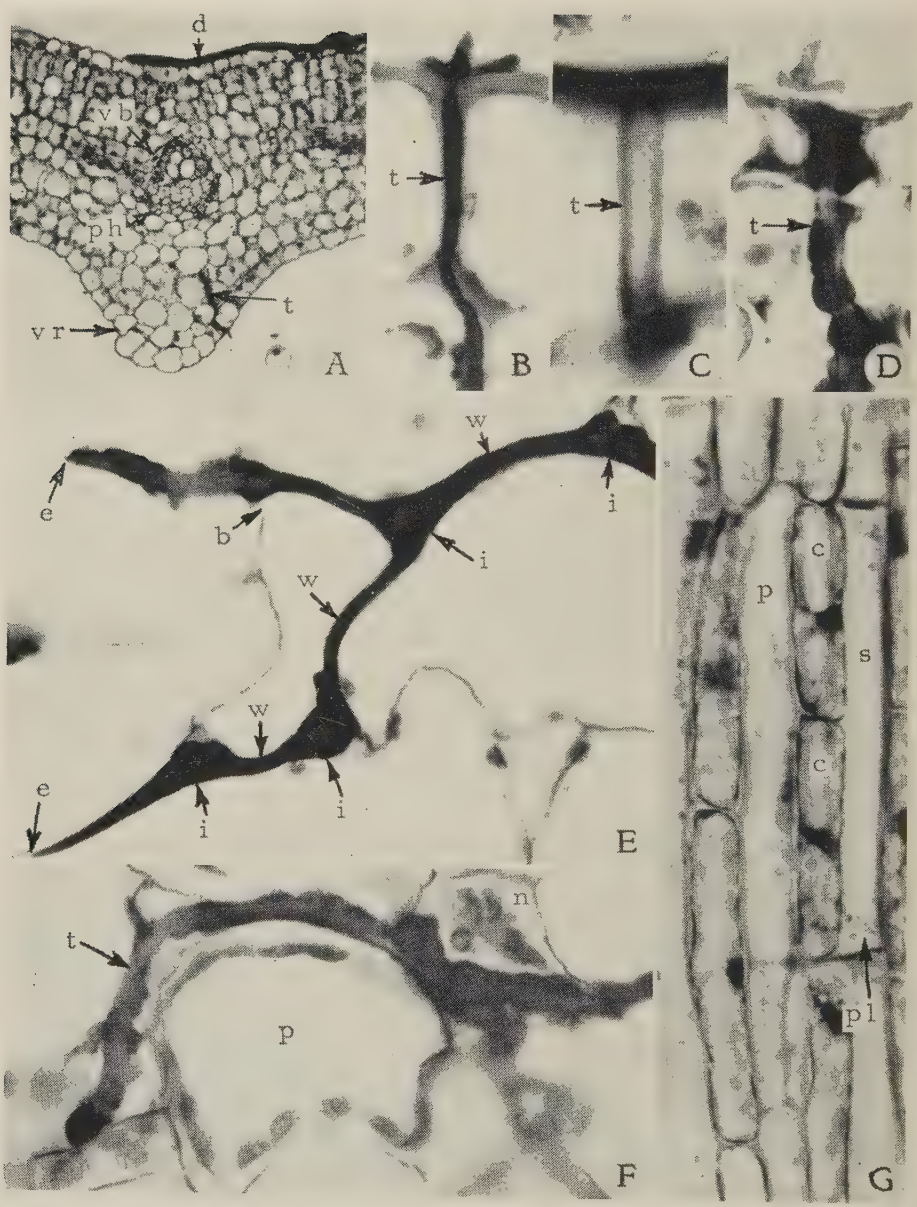

Fig. 1. *A*, transection of leaf showing the track *(t)* of saliva sheath formed during an approximately 4-minute penetration within the parenchyma of the vein rib *(vr)*. The track is directed toward the vascular bundle *(vb)* the phloem of which is located at *ph*. Part of the India ink dot *(d)* appears on the upper surface of the leaf. *B*, part of a saliva track *(t)* in intercellular position in the epidermis, shown in sectional view of the wall. *C*, part of a saliva track *(t)* in intercellular position in the epidermis, shown in surface view of the wall. *D*, saliva track *(t)* that passed through an epidermal cell. *E*, part of a saliva track within vein-rib parenchyma. It shows swellings in the intercellular spaces *(i)* and is smooth within the cell wall *(w)*. At left, above, one arm of the track has broken the wall of a parenchyma cell at *b*. Ends of the two arms of the track are indicated by letters *e*, one within cell wall

fornia. The other method consisted of placing individual insects on leaves and letting them terminate the penetration naturally. The aphid, placed in the middle of the leaf blade, on its upper surface, usually moved to the lower side before attempting a penetration. Occasionally it punctured the leaf through the upper surface. The penetration periods were timed with a stop watch while the insects were observed through a magnifying lens. To locate the stylet punctures for processing for microscopy, a dot of India ink was placed gently on the leaf surface opposite the insect as soon as the duration of the penetration exceeded one minute. The ink dot served as a guide not only for collecting the material but also for searching for the saliva track in the sections (fig. 1, *A*).

An additional experiment was carried out to study the insect's habit with regard to changing the place of penetration and the duration of a single penetration. An aphid was placed in the middle of the upper side of a leaf and was observed through a magnifying glass. The penetrations were timed with a stop watch. When the aphid wandered off the plant, or when it had made a penetration of more than two hours, the observation was terminated.

PENETRATIONS BY GROUPS OF APHIDS

In this experiment groups of insects were given access to feeding on a leaf for 5, 10, 20, 30, and 40 minutes, and for 1 and 2 hours. Sample sections of leaves exposed to the insects for 5 minutes (12 leaf pieces) and 10 minutes (4 leaf pieces) revealed no saliva sheaths and these sections, therefore, were not studied further. The longer-lasting penetrations were analyzed in detail.

The penetrations were characterized as shallow, medium, and deep, depending on the number of cells through which the track marked by the saliva sheath extended. The shallow penetrations were 1 to 3 cells in depth (including the epidermal cells), the medium, 4 cells, the deep, 5 cells or more. The deep penetrations had tracks long enough to reach the phloem of the intermediate veins in the mesophyll but not necessarily the smaller, more deeply imbedded bundles, or the larger bundles associated with vein ribs. None of the shallow or medium penetrations had reached the phloem.

The shallow and medium penetrations were excluded from the calculations of the per cent penetrations that reached the various tissues (table 1) because it was assumed that the short tracks may have resulted from short-time probing penetrations. The combined numbers and per cents of the shallow and medium penetrations in the three time groups in table 1 were as follows: for the 20 to 40-minute period, 9 (34.6 per cent); for the 1-hour period, 9 (26.5 per cent); for the 2-hour period, 7 (12.3 per cent); for all time groups combined, 25 (21.4 per cent). The penetration periods of 20 to 40 minutes were combined because of the small number of penetrations available for each. It should be remembered that the timings of penetrations given in table 1 are only approximate because the individual penetrations were not timed in this experiment.

(below), the other in the lumen of a cell (above). *F*, part of a saliva track *(t)* in mesophyll. It is intracellular at *n*, and mostly in intercellular space elsewhere. The pierced cell at *n*, is partly necrosed, the parenchyma cell at *p*, in contact with the track, is normal. *G*, longitudinal section of phloem showing a phloem-parenchyma cell *(p)*, companion cells *(c)*, and a sieve element *(s)* with plastids *(pl)*. (*A*, × 145; *B*, *C*, × 1,530; *D*, *E*, *F*, × 990; *G*, × 515.)

The third, fourth, and fifth columns in table 1 show that, among the deep penetrations, 43.5 per cent reached the phloem. Those that did not reach this tissue were either directed toward the vascular bundles without reaching them, or entered the parenchyma between the bundles. If the penetrations are classified according to whether or not they were directed toward the vascular bundles, almost 70 per cent fall into the class that did reach the phloem or could have done so if they had been extended far enough. Only one out of 92 penetrations ended in the xylem. This was one of the few penetrations that were initiated on the upper side of the leaf. Of the three other penetrations that were initiated on the upper side, two ended in the phloem and one in parenchyma between the vascular bundles.

TABLE 1

ANALYSIS OF DEEP PENETRATIONS BY STYLETS OF *MYZUS PERSICAE* PLACED ON SUGAR BEET LEAVES IN GROUPS, WITH ACCESS TIME ARTIFICIALLY INTERRUPTED

Access time	Number of penetrations	Per cent of penetrations that reached the various tissues			Per cent of penetrations that were directed toward:	
		Parenchyma	Xylem	Phloem	Parenchyma	Vascular bundle
20–40 min	17	76.5	....	23.5	35.3	64.7
1 hr	25	60.0	....	40.0	36.0	64.0
2 hr	50	46.0	2.0	52.0	26.0	74.0
Total and per cents of total	92	55.4	1.1	43.5	30.4	69.6

INDIVIDUALLY-TIMED PENETRATIONS

Table 2 records the study on the duration, sequence of durations, and number of penetrations for individual *M. persicae* left undisturbed on sugar beet leaves. As can be seen, the feeding activities were highly variable among the aphids, and most penetrations lasted less than 1 minute. In the study detailed in table 3 a larger number of penetrations were maintained for several minutes. By carrying out a series of 5-minute observations, Sylvester (1954) also noted a variability in the feeding activities of *M. persicae* (on *Brassica juncea* plants infected with *Brassica nigra* virus). We observed that the first two or three penetrations were initiated abruptly, whereas the later ones—even those that were of short duration and were preceded and followed by short-time penetrations—were preceded by light, rapid taps on the leaf surface with the tip of the insect's beak.

Table 3 details the observations on the penetration tracks formed by individually-timed insects. The penetrations listed in the first column were made either singly by one aphid or several in succession by one aphid. In this experiment the largest number of penetrations by one aphid was four. Of these four, one was not recognized in the sections; the other three were numbers 20, 26, and 41 in table 3. The first three penetrations by this one aphid were of relatively short duration and were made in the following sequence: No. 26, 9 minutes; missing number, 13 minutes; No. 20, 6 minutes. Then fol-

TABLE 2

DURATION OF INDIVIDUAL PENETRATIONS OF SUGAR BEET LEAVES BY STYLETS OF *MYZUS PERSICAE*

Penetration number	Aphid number										
	1	2	3	4	5	6	7	8	9	10	11
1	27 sec	20 sec	20 sec	15 sec	17 sec	16 sec	17 sec	13 sec	26 sec	13 sec	15 sec
2	27 sec	36 sec	1 min 20 sec	15 sec	15 sec	23 sec	16 sec	20 sec	28 sec	4 min 43 sec	15 sec
3	1 min 19 sec	8 min 29 sec	2 min 37 sec	20 sec	10 sec	14 sec	47 sec	>2 hr	6 min 16 sec	16 sec	17 sec
4	23 sec			21 sec	13 sec	7 min 8 sec	29 sec		25 sec	17 sec	15 sec
5	13 sec			12 sec	13 sec	13 sec	25 sec		32 sec	10 min 23 sec	6 min 29 sec
6	13 sec			1 min 10 sec	1 min 32 sec	14 sec	12 sec		54 sec	19 sec	13 sec
7	17 sec			16 sec	13 sec	12 sec	12 sec		17 sec	15 sec	15 sec
8	19 sec			8 min 50 sec	18 sec	10 sec	9 sec		16 sec	15 sec	22 sec
9	13 sec			18 sec	13 sec	17 sec	2 min 50 sec			15 sec	5 min 12 sec
10	10 sec			28 sec	18 sec	14 sec	4 min 25 sec			16 sec	9 sec
11	15 sec			2 min 40 sec	12 sec	18 sec	>2 hr			7 min 28 sec	12 sec
12	8 min 15 sec*			>2 hr	12 sec	10 sec				10 sec	10 sec
13					15 sec	23 sec				5 sec	10 sec
14					29 sec	12 sec					1 min 2 sec
15					14 sec	17 sec					40 sec
16					14 sec	12 sec					13 sec
17					15 sec	19 sec					24 sec
18					1 min 38 sec						13 sec
19					11 sec						58 sec
20					15 sec						14 sec
21					8 sec						
22					14 sec						
23					13 sec						
24					11 sec						
25					9 sec						

* Observation was discontinued before the aphid naturally terminated the penetration.

TABLE 3

INDIVIDUALLY-TIMED AND NATURALLY-TERMINATED PENETRATIONS OF SUGAR BEET LEAVES BY STYLETS OF *MYZUS PERSICAE*

Penetration number	Duration of penetration	Depth of penetration			Tissues reached		Direction of penetration	
		Shallow	Medium	Deep	Parenchyma	Phloem	Parenchyma	Vascular bundle
1	1–2 min	+			+			+
2		+			+			+
3		+			+			+
4		+			+			+
5		+			+		+	
6		+			+		+	
7		+			+			+
8	2–3 min	+			+			+
9		+			+		+	
10		+			+			+
11		+			+		+	
12		+			+		+	
13	3–4 min	+			+			+
14				+	+			+
15		+			+			+
16				+		+*		+
17	4–5 min			+	+		+	
18	5–6 min			+		+		+
19			+		+		+	
20			+		+			+
21			+		+		+	
22	7–8 min			+	+		+	
23				+	+			+
24	9 min			+	+		+	
25				+	+		+	
26				+	+			+
27	10 min			+	+			+
28	11–15 min			+	+			+
29				+	+		+	
30				+	+			+
31	15–20 min			+	+			+
32				+	+			+
33				+	+			+
34				+		+		+
35				+	+		+	
36	30 min			+	+			+
37				+		+		+
38				+		+		+
39	1–2 hr			+		+		+
40				+		+		+
41	>2 hr†			+		+*		+
42				+		+		+
43				+		+		+
44				+		+		+
45				+		+		+
46				+		+		+
47				+		+		+
48				+		+		+
49				+		+		+
Totals (49)		14	3	32	33	16	14	35
Per cent		..	..	..	67.3	32.7	28.6	71.4
Per cent for deep penetrations only		..	..	..	50.0	50.0	21.9	78.1

* Terminated in xylem but passed through phloem.
† The insect was removed after 9.5 hr of feeding because it produced young aphids.

lowed penetration No. 41, which lasted 9.5 hours, after which the insect was destroyed because it entered vivipary. As shown in table 3, the last penetration reached the vascular tissue.

Table 3 as a whole clearly indicates that short-time penetrations rarely reach the phloem. Penetrations 16 and 18 constitute the two notable exceptions. These two penetrations showed no unusual features in the relation of the saliva sheath to the host tissues. Penetration 16 was made in a somewhat older leaf with large intercellular spaces, penetration 18 in a younger leaf with small intercellular spaces. In both, the track was partly intercellular and partly intracellular. Beginning with the second 30-minute penetration (No. 37), the feeding tracks were consistently terminated in the phloem. The proportion of terminations in the phloem for the deep penetrations is slightly higher in table 3 than in table 1. Statistically, however, the difference is not significant: adjusted $X^2 = 0.71$; d.f. = 1; $P = 0.39$.

HISTOLOGIC ASPECTS OF PENETRATIONS

The relation of the saliva sheaths of aphids to the host tissues has been studied by several workers (see "Discussion," p. 526). The conclusions reached by most workers regarding the manner of entry of the mouth parts into a leaf and their passage and direction through the tissues were confirmed in the present study; but we failed to recognize the penetration of sieve elements.

It is obvious that *M. persicae* feeding on sugar beet leaves does not seek out the stomata. Where penetrations occurred in a stomatal area, the track was made through cell walls or through cells; in one instance it was seen traversing the guard cell itself. Not a single clear evidence of penetration through a stomatal pore was found. Entry through the epidermal cell wall, that is, an intercellular entry (fig. 1, *A–C*), appears to be more common than one through an epidermal cell (fig. 1, *D*). The characteristic saliva deposit on the surface of the epidermis (fig. 1, *B, D*; cf. also Hoof, 1958, and Smith, 1926) was noted in many sections.

The path through the mesophyll, as well as through the parenchyma (fig. 1, *A, E*) and collenchyma of the vein ribs, was partly or entirely intercellular. If intercellular spaces were present in the tissue, the saliva sheath showed enlargements in those spaces (fig. 1, *E* at *i*). Piercing of cells was noted more often in younger than in somewhat older leaves. (All leaves used were relatively young.) Several of the tracks were branched (e.g., fig. 1, *E*).

In some sections a disorganization of protoplasts of the pierced cells was recognized. The cells sometimes showed an increase in density of cytoplasm (fig. 1, *F* at *n*) and a degeneration of plastids, at other times an advanced stage of necrosis (fig. 1, *D*, epidermal cell). The necrosed material was stained red, but differed from the red-staining saliva sheath in having a somewhat yellowish hue combined with the red. In some pierced cells disintegration phenomena were not recognized (fig. 1, *E*, at left above), possibly because of technical imperfections. No evidence was found that cells located next to a track, but not pierced by stylets, were affected (fig. 1, *F* at *p*).

The saliva sheath showed the characteristics described by Hoof (1958). The sheath was smooth when enclosed between cell walls (fig. 1, *B, C, E* at *w*), but it was uneven when located in intercellular spaces (fig. 1, *E* at *i*) or within cells (fig. 1, *F* at *n*). No difference in thickness was found between

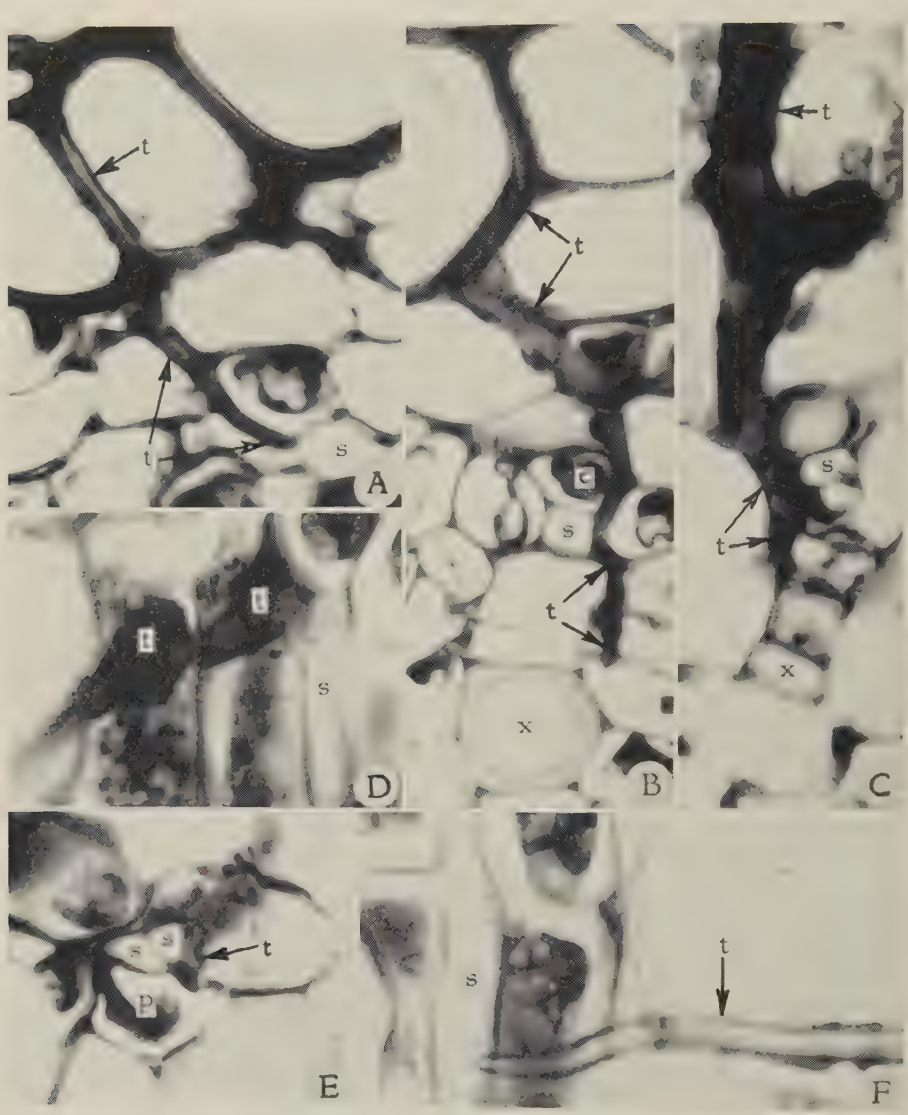

Fig. 2. *A*, part of a saliva track *(t)* in phloem in transection. It is intercellular in position, and directed toward a sieve element *(s)*. *B*, part of a saliva track *(t)* in phloem in transection. It is intercellular in position, passes through the phloem in contact with a sieve element *(s)* and a companion cell *(c)*, and terminates in procambium. *C*, part of a saliva track *(t)* in mesophyll passing, intercellularly, next to a minor vein in contact with a sieve element *(s)*. This track continued beyond the bundle almost through the entire thickness of the mesophyll. *D*, part of a saliva track in phloem in longitudinal section with two swellings *(t)* produced in the two parenchyma cells that were pierced. A sieve element occurs at *s*. The very tip of the track was bent away from the plane of the photograph. *E*, part of a saliva track *(t)* in phloem in transection. The tip of the track is within a parenchyma cell and has dented the wall of another parenchyma cell *(p)*. Two sieve elements appear at *s*. *F*, part of a saliva track *(t)* in phloem in longitudinal section. It is intercellular in position and is superimposed over a parenchyma cell and a sieve element *(s)*. The xylem is indicated at *x* in *B* and *C*. (All × 1,530.)

sheaths that reached the phloem and those that did not. However, many of the sheaths formed in the experiment with individual aphids (table 3) were noticeably thicker than others (e.g., figs. 1, *A*, and 2, *C*).

A special effort was made to determine the relation of the feeding tracks to the sieve elements. The total number of penetrations that reached the phloem in both experiments was 56. Of these, only 33 showed the sheath and the sieve elements in such a way that their spatial relation could be determined under an oil-immersion lens. Such a determination is by no means simple, especially if the sieve elements are narrow. The sieve elements of the sugar beet are rather narrow cells, particularly in the minor veins (Esau, 1960) in which the feeding tracks so often terminate. However, the characteristic clearness of the contents of the sieve elements and the presence of sieve-tube plastids (fig. 1, *G*) help to identify these cells even if the vascular bundles are cut on a bias.

Among the 33 tracks examined with regard to their course in the phloem, not a single one terminated in a sieve element. The closest to such a termination is shown in figure 2, *A*. Here, the track (*t*) is intercellular, and ends just outside the wall of a sieve element (*s*). In several views the track was seen in contact with sieve elements. In figure 2, *B*, for example, the track passed intercellularly through the phloem directly next to a sieve element (*s*) and a companion cell (*c*), and ended in the procambium. In figure 2, *C*, the track is seen flanking, intercellularly, a small vascular bundle, again in contact with a sieve element (*s*). This particular track continued beyond the bundle in the next section, passing almost through the entire mesophyll. In several views the track was seen to have entered a parenchyma cell next to a sieve element. Although none of these tracks was satisfactory for photomicrography, two are shown. In figure 2, *D*, the track (*t*) passed through one parenchyma cell and entered a second, which was located next to a sieve element (*s*). (The figure does not show the very end of the saliva sheath because it was bent away from the plane of photography.) In figure 2, *E*, the end of the sheath appears within a parenchyma cell next to two sieve elements, and its point has dented the wall of another parenchyma cell (*p*). (The shrinkage of the protoplast in the parenchyma cell *p* appears to be a result of poor fixation.) In one section the saliva sheath had traversed a phloem parenchyma cell and entered a companion cell. Both cells had gum-like necrotic material next to the saliva sheath.

A view that required extra care in interpretation is shown in figure 2, *F*. It gives the impression that the track ends within a sieve element. However, the relatively smooth appearance of the sheath suggests that it is in an intercellular position. There is no evidence of a break in the wall of the sieve element such, for example, as is recognizable in the wall of the pierced parenchyma cell at *b* in figure 1, *E*. The view in figure 2, *F*, actually shows the saliva track in an intercellular position superimposed over a parenchyma cell and a sieve element.

DISCUSSION

As judged by the histological aspects of the penetration of sugar beet leaves by the stylets of *Myzus persicae*, the insect could release the beet yellows virus into and pick it up from both the phloem and the parenchyma outside the phloem; it may pierce cells in the phloem and also those outside that tissue

The conclusion of Hoof (1958), that aphids can pick up a virus from the inside of a cell wall, cannot be properly evaluated on the basis of the present study. There is no question, however, that some saliva sheaths are entirely intercellular in position. Another problem yet to be solved is whether or not the degeneration of the protoplasts of the pierced cells has any effect upon virus transmission.

Bennett's (1960) and Roberts' (1940) observations that penetrations of longer duration, that is, penetrations which are likely to reach the phloem, are more effective in beet yellows virus transmission than are the short-time penetrations, and the present confirmation that short-time penetrations rarely reach the phloem, support the concept that penetration of the phloem is important for the success of infection. When placed in the phloem, the beet yellows virus may be expected to be carried through the plant in sieve elements, although the present study furnishes no direct evidence that the insect punctures the sieve elements in feeding on the phloem.

The question whether or not the aphid finds the vascular bundles simply by random probing, as has been suggested for the jassids by Day *et al.* (1952), cannot be answered as yet. The preference of the insect for the lower side of the leaf, and the frequent selection of veins suggest some sense of direction, but the observation that only about half of the deep penetrations reach the phloem points toward the possibility that the sense of direction is somewhat limited. A related question is concerned with whether the insect feeds only in the phloem tissue or also in the ground parenchyma. Possibly the shallow tracks, especially those that appear to be entirely intercellular, result from probing penetrations and involve no feeding; but there is also the high percentage of deep and prolonged penetrations not ending in the phloem that cannot be properly interpreted at the present state of our knowledge of the feeding habits of aphids.

The tracks formed in host tissues by aphids and many other insects in their search for food have been repeatedly investigated with and without reference to the transmission of viruses. Only one aspect of these studies is reviewed here: whether or not conclusive evidence has been presented that, in penetrating the phloem, an insect actually punctures a sieve element. The assumption is generally made that insects feeding on the phloem do tap the sieve elements. For the aphids specifically it is postulated that they do not suck the contents of sieve elements, but depend almost entirely on the pressure of the sieve-element contents, which forces the sap up the food canal of the stylet (Mittler, 1957). The ingenious experiments involving the severance of aphids from their stylets during the process of feeding, and the collecting of sap exuded from the stylets have enabled workers to carry out some highly significant studies on the phloem sap; and the composition of the exudate suggests that it could have originated in sieve elements (e.g., Mittler, 1957, 1958; Weatherley *et al.*, 1959). With reference to these experiments, as well as with regard to the transmission and translocation of viruses, the determination of the cell tapped by the insect is of great interest.

Published papers dealing with feeding tracks of various insects, reviewed in connection with the present study, reveal that many authors have submitted illustrations clearly demonstrating penetration of the phloem tissue (e.g., Dykstra and Whitaker, 1938; Leonhardt, 1940; Smith, 1926; Tate,

1937). With regard to the puncturing of sieve elements, however, we are given either statements (e.g., Mittler, 1957; Lindemann, 1949; Weatherley *et al.*, 1959), or idealized drawings (e.g., Horsfall, 1923; Rawitscher, 1933–34), or inadequate photomicrographs. In the photomicrograph by Romell (1935), supposedly depicting punctured sieve elements, the identity of these elements is not obvious in the picture, and they are said to be "filled with cytoplasm," a feature not typical of mature sieve elements. The low-power photomicrographs of sections cut on bias reproduced in the paper by Franke-Grosmann (1937) are even less informative regarding cells penetrated by the aphid (*Lachnus pichtae*) in its host (*Abies*). The drawings of Hargreaves (1915) and Pollard (1955), depicting the feeding of a white fly larva, show the stylets of the insect ending in a bundle-sheath cell. Pollard states that "the stylet penetrates a sieve-tube," but his drawing, obviously, does not confirm this conclusion. Photomicrographs most closely suggesting penetration of sieve elements by insect (aphid) stylets appear in Mittler's thesis (1954). An entirely certain identification of the cells in which the stylets end is not possible from these illustrations, but one of them shows the tip of the stylet bundle located in an elongated cell, and this tip is free of saliva.[6]

This absence of saliva at the tip of the stylets appears to be significant in relation to the present study in which only saliva sheaths were examined. Conceivably, the insect projects the stylets beyond the saliva sheath during the actual feeding process and penetrates the sieve element without leaving any easily detectable puncture. Such projection of mouth parts beyond the saliva sheath was observed in jassids feeding on a solution through a plastic membrane (Day *et al.*, 1952). In fact, deposition of saliva into the sieve element could possibly interfere with the feeding process by interrupting the flow of sap from the sieve element.

SUMMARY

By reference to saliva sheaths left by *Myzus persicae* in sugar beet leaves, over 150 penetrations were studied with regard to their position in the leaf tissues. Some of these penetrations were made by aphids placed in groups upon single leaves and then killed after having had access to the leaves for periods of various lengths. The others were obtained by placing individual insects upon a leaf and timing naturally-terminated penetrations. When penetrations lasted only a short time they usually were shallow. Penetrations of longer durations produced deeper punctures, and of these approximately 50 per cent terminated in the phloem tissue, the others in the mesophyll or other parenchyma. Within the phloem, the saliva sheath may be entirely intercellular or may traverse or terminate in phloem-parenchyma cells or companion cells. The sheath was seen in contact with sieve elements in intercellular position. Outside the phloem the sheath may be entirely intercellular or may penetrate epidermal or parenchyma cells.

[6] Since this review was written, Dr. Martin H. Zimmermann (1961) of Harvard University has succeeded in obtaining convincing photomicrographs showing the stylets of a large aphid, *Longistigma caryae*, ending in a sieve tube of *Tilia*. As in Mittler's (1954) figures, the stylet ends are free of saliva in those photomicrographs.

LITERATURE CITED

Bennett, C. W.
1960. Sugar beet yellows disease in the United States. U. S. Dept. Agr. Tech. Bul. **1218**.

Day, M. F., H. Irzykiewicz, and A. McKinnon
1952. Observations on the feeding of the virus vector *Orosius argentatus* (Evans), and comparisons with certain other jassids. Aust. Jour. Sci. Res. Ser. B. **5**: 128–42.

Dykstra, T. P., and W. C. Whitaker
1938. Experiments on the transmission of potato viruses by vectors. Jour. Agr. Res. **57**: 319–34.

Esau, K.
1960. Cytologic and histologic symptoms of beet yellows. Virology **10**: 73–85.

Franke-Grosmann, H.
1937. Zur Kenntnis der Läuseschäden an Weisstanne *(Abies pectinata)*. Tharandt. Forstl. Jahrb. **88**: 1050–82.

Hargreaves, E.
1915. The life history and habits of the greenhouse whitefly *(Aleyrodes vaporariorum* Westd.) Ann. Appl. Biol. **1**: 303–34.

Hoof, H. A. Van
1958. Onderzoekingen over de biologische overdracht van een non-persitent virus. [An investigation of the biological transmission of a non-persistent virus.] Wageningen. (Thesis.) 96 pp.

Horsfall, J. L.
1923. The effect of feeding punctures of aphids on certain plant tissues. Penn. Agr. Exp. Sta. Bul. **182**: 96 pp.

Leonhardt, H.
1940. Beiträge zur Kenntnis der Lachniden, der wichtigsten Tannenhonigtauerzeuger. Ztschr. Angew. Ent. **27**: 208–72.

Lindemann, C.
1949. Beitrag zur Ernährungsphysiologie der Blattläuse. Ztschr. Vergl. Physiol. **31**: 112–33.

Mittler, T. E.
1954. The feeding and nutrition of aphids. University of Cambridge (Unpublished thesis).
1957. Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae). I. The uptake of phloem sap. Jour. Exp. Biol. **34**: 334–41.
1958. Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. Jour. Exp. Biol. **35**: 74–84.

Pollard, D. G.
1955. Feeding habits of the cotton whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). Ann. Appl. Biol. **43**: 664–71.

Rawitscher, F.
1933–34. Wohin stechen die Pflanzenläuse? Ztschr. Bot. **26**: 145–68.

Roberts, F. M.
1940. Studies on the feeding methods and penetration rates of *Myzus persicae* Sulz., *Myzus circumflexus* Buckt., and *Macrosiphum gei* Koch. Ann. Appl. Biol. **27**: 348–58.

Romell, L.-G.
1935. Savhonung. [Miel de sève.] Svensk Bot. Tidskr. **29**: 391–406.

Sass, J. E.
1958. Botanical microtechnique. (3d ed.) Ames, Iowa: Iowa State College Press. 228 pp.

Smith, K. M.
1926. A comparative study of the feeding methods of certain Hemiptera and of the resulting effects upon the plant tissue, with special reference to the potato plant. Ann. Appl. Biol. **13**: 109–39.

SYLVESTER, E. S.
1954. Aphid transmission of nonpersistent plant viruses with special reference to the *Brassica nigra* virus. Hilgardia **23**(3): 53–98.
1956. Beet mosaic and beet yellows virus transmission by the green peach aphid. Amer. Soc. Sugar Beet Technol. Jour. **9**: 56–61.
TATE, H. D.
1937. Method of penetration, formation of stylet sheath and source of food supply of aphids. Iowa State Coll. Jour. Sci. **11**: 185–206.
WEATHERLEY, P. E., A. J. PEEL, and G. P. HILL
1959. The physiology of the sieve tube. Preliminary experiments using aphid mouth parts. Jour. Exp. Bot. **10**: 1–16.
ZIMMERMANN, M. H.
1961. Movement of organic substances in trees. Science **133**: 73–79.

4*m*-3,'61(B6650)LL